UNIVERSITY OF ALBERTA

NMR SPECTROSCOPY IN THE ASSESSMENT OF TUMOR RESPONSE IN COMBINATION PHOTODYNAMIC, HYPOXIC AND CYTOTOXIC THERAPY.

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A thesis submitted to The Faculty of Graduate Studies and Research in partial fulfillment of the requirement for the Degree of:

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In

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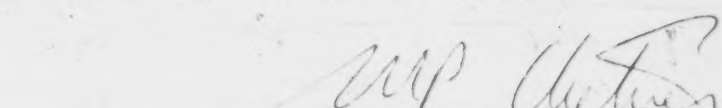

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NMR Sepctroscopy in the Assessment of Tumor Response in Combination Photodynamic, Hypoxic and Cytotoxic Therapy.

Submitted by:

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in partial fulfillment of the requirements for the degree of:

Masters of Science

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J.C. Russell (Committee member)

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THIS THESIS IS DEDICATED TO MY WIFE, BONNIE LYNN.

FOR HER UNDYING LOVE, SUPPORT AND PATIENCE

I AM ETERNALLY GREATFUL

ABSTRACT:

Photodynamic therapy (PDT) is a new approach for the treatment of cancer, and the mechanisms by which PDT exerts its tumoricidal effect have been examined from many perspectives. PDT presumably causes tumor destruction by direct cellular cytotoxicity as well as by induction of hypoxia and tumor ischemia secondary to microvascular destruction. Tumor hypoxia correlates positively with changes in cellular phosphate metabolism as detected *in-vivo* by ^{31}P NMR spectroscopy.

In the first experiment of this thesis, ^{31}P NMR spectra of Dunning -H and -AT tumors were obtained before and after PDT (Healux H628), at various light doses. No significant changes were seen in the phosphorous spectra after PDT at total light doses from 100 to 2000 J/cm^3. A second experiment analyzing the delay in Dunning -AT tumor regrowth, failed to demonstrate any significant difference in tumor response following PDT either 4 hours or 24 hours after Photofrin II administration. However, both treatment groups showed a statistically significant delay in tumor regrowth compared to controls. A third experiment comparing Misonidazole (MISO) and two newer hypoxic cytotoxic drugs, RSU-1069 and SR-4233, each used in combination with PDT (Healux H628, 1000 J/cm^3), demonstrated that all three hypoxic cytotoxic agents potentiated the PDT response when compared to untreated controls. No significant difference was noted between the tumor response of the three treatment groups but SR-4233 was more systemically toxic to the animals.

A final pair of experiments, using the Dunning -AT tumor, compared the photosensitizing potential of Healux H628 and Photofrin II. Combination MISO and PDT (1000 J/cm^3) in tumors sensitized by Photofrin II caused a significantly longer delay of tumor regrowth than seen in similarly treated tumors sensitized by Healux H628. Significant differences in ^{31}P NMR spectra of tumors after PDT (2000 J/cm^3) sensitized by Photofrin II were noted when no such changes were seen after PDT sensitized by Healux H628. The results of these two experiments suggests that Photofrin II is a superior photosensitizer to Healux H628.

MY MOST SINCERE HEARTFELT THANKS GOES TO ALL OF MY MENTORS FOR THE WISDOM, KNOWLEDGE, PATIENCE AND UNDERSTANDING THAT THEY HAVE TRIED TO IMPART TO ME DURING MY MEDICAL TRAINING.

TABLE OF CONTENTS:

	PAGE
(A) INTRODUCTION.	
Photodynamic Therapy.	1
Hypoxic Cytotoxic Adjuvants.	8
Hypothesis.	13
(B) MATERIALS AND METHODS.	
Animal Model.	16
Tumor Model.	16
Animal Anesthetic Technique	18
Tumor Transfer Technique	18
Tumor Measurement.	19
Photosensitizers.	19
Laser Phototherapy.	21
Specific Experimental Protocols.	21
(C) RESULTS	27
(D) DISCUSSION.	35
(E) CONCLUSION.	53
(F) FIGURES.	55
(G) TABLES.	60
(H) REFERENCES.	71

LIST OF FIGURES:

Figure 1: Diagram of experimental design for interstitial PDT.

Figure 2: Graph comparing tumor growth delay after PDT sensitized with either Healux H628 or Photofrin II.

Figure 3: Graph comparing tumor growth delay after PDT combined with either Misonidazole, RSU-1069 or SR-4233.

Figure 4: Graph comparing tumor growth delay after PDT sensitized either 4 hours or 24 hours prior to photoirradiation.

Figure 5: Graph comparing the relative amounts of phosphorous signal after PDT photosensitized with either Healux H628 or Photofrin II.

LIST OF TABLES:

Table 1: Control ^{31}P NMR Spectra, Dunning -H tumor.

Table 2: Control ^{31}P NMR Spectra, Dunning -H tumors listed by tumor volume.

Table 3: Comparison of control ^{31}P NMR spectra, seven smallest and five largest Dunning -H tumors.

Table 4: Treatment ^{31}P NMR spectra, Dunning -H tumors.

Table 5: Control ^{31}P NMR Spectra, Dunning -AT tumor.

Table 6: Control ^{31}P NMR Spectra, Dunning -AT tumors listed by tumor volume.

Table 7: Comparison of control ^{31}P NMR spectra, six smallest and five largest Dunning -AT tumors.

Table 8: Treatment ^{31}P NMR spectra, Dunning -AT tumors.

Table 9: Comparison of control ^{31}P NMR spectra, Dunning -H and Dunning -AT tumors.

Table 10: Comparison of ^{31}P NMR spectra from Dunning -AT tumors before PDT sensitized with either Healux H628 or Photofrin II.

Table 11: Comparison of ^{31}P NMR spectra from Dunning -AT tumors after PDT sensitized with either Healux H628 or Photofrin II.

INTRODUCTION:

Photodynamic Therapy:

Photodynamic therapy (PDT) is the use of light to excite a photosensitizing agent which, in the presence of oxygen, produces cytotoxic by-products which are lethal to both histologically normal and neoplastic cells.

One of the earliest references to PDT was by Marcacci who, in 1888, noted that quinine, in combination with light, had an inhibitory effect on plant growth.[1] Raab in 1900 destroyed paramecia with a combination of light, acridine orange and eosin dyes.[2] Possibly the first clinical use of PDT was reported in 1903 by Von Tappeiner and Jesionek. They "destroyed skin tumors" with the combination of eosin and white light.[3] In 1924 Policard documented red fluorescence of tumors under ultraviolet (uv) light and he attributed this fluorescence to porphyrins intrinsic to the tumors.[4] Auler and Banzer noted fluorescence of rat tumors after systemic injection of hematoporphyrin and exposure to light.[5] In 1961, the diagnostic work of Lipson and Baldes demonstrated red fluorescence of bronchial and esophageal malignancies, commenting on the "increased affinity" of hematoporphyrin derivative (HpD) for neoplastic cells.[6] The first attempted use of HpD in photodynamic therapy (PDT) was reported by Lipson in 1966.[7]

To understand this theraputic use of HpD as a photosensitizer, it is useful to examine some of its qualities and properties. Hematoporphyrin derivative

(HpD) is a mixture of porphyrins chemically derived from hematoporphyrin (Hp).[8] Hematoporphyrin is acetylated and then dissolved in dilute sodium hydroxide or mildly acidic saline producing a mixture of porphyrin derivatives which are active as both photosensitizers and tumor fluorescence localizers.[9] In 1981, Dougherty used high-pressure, liquid chromatography, mass spectroscopy and NMR spectroscopy to identify various components of HpD. High pressure liquid chromatography yielded 4 fractions.[8] The first fraction (I) contained hematoporphyrin (Hp); the second fraction (II) contained hydroxyethylvinyldeuteroporphyrin (HVD); the third fraction contained protoporphyrin (PP) and what is thought to be the active component of HpD, di-hematoporphyrin ether or ester (DHE); and the last fraction contained metalloporphyrin possibly produced by metal contamination from the chromatography column.[10] The active component of this mixture has tentatively been identified by NMR spectroscopy as bis-1-[3(1-hyroxy-ethyl) deuteroporphyrin-8-yl] ethyl ether.[11] There is some debate as to whether that compound is truly an ether or an ester but it comprises approximately 45% of HpD.[9,11] Of all four fractions of HpD the fraction containing DHE demonstrated the highest level of absorption and fluorescence.[10] It also demonstrated the most cytotoxicity based on cell culture experiments.[10] In fact, the cytotoxicity of HpD was the same as the cytotoxicity of only the third fraction (i.e., DHE) suggesting that all of HpD's cytotoxic effects were due to its third fraction.[10]

HpD can be excited by light at many different wavelengths. The most intense excitation occurs at the "Soret band".[12] This is violet light at 400-410

nm. HpD excitation can also occur at 500-505 nm, 535-540 nm, 565-575 nm and 620-635 nm but as the wave length increases, the intensity of excitation decreases proportionally. The longer the wavelength of light the greater the tissue penetration. For PDT, one must use light in the visible red spectrum or longer, in order to ensure an adequate depth of penetration by light into tissues of greater than 5 mm thickness.

The disadvantage of HpD as a photosensitizer is that despite its peak of excitation at 630 nm, its ability to transfer photoenergy is 10-fold less efficient, at this wavelength, than at 410 nm.[12] Despite this weak excitation peak at 630 nm, the majority of *in-vivo* work with PDT utilizes 630 nm red light for inducing the photochemical reaction leading to its cytotoxic effect. This is done to take advantage of the greater tissue penetration afforded by light of a longer wavelength.

In 1972 Diamond successfully treated mouse glioma tumors with PDT *in-vivo* and also killed tumor cells while they were in culture.[13] The first clinical urologic application of PDT was reported by Kelly in 1975.[14] He transplanted human bladder tumors into immunosuppressed mice and effected a cure with HpD and red spectrum light. In 1976 Kelly and Snell reported the first successful clinical use of PDT in treatment of transitional cell carcinoma.[15] Since 1976, there have been several clinical studies using PDT. Cutaneous malignancies, including basal cell carcinoma, squamous cell carcinoma, malignant melanoma and Bowen's disease have been successfully treated with PDT.[9] PDT has also produced a good clinical response in obstructing

carcinoma of the esophagus and lung.[9] Transitional cell carcinoma of the bladder has been successfully treated by PDT, as reported in several clinical trials.[9] The use of PDT in treatment of gynecologic malignancies and tumors of the head and neck has also been well documented.[9]

A review of the photochemical reaction which occurs in PDT was published by Foote in 1984.[16] Photodynamic therapy involves a sequence of photochemical reactions where light energizes or excites a photosensitizing agent (i.e., HpD) and then the energized photosensitizing agent interacts in different ways with various organic molecules, with in cells, to elicit its phototoxicity.[16] The first step in the process involves the transfer of light energy to a photosensitizer and results in its transformation to an excited electronic state.[16,17] HpD exists in two excited states, a singlet form where all the electron spins are paired, and a triplet form where there are two unpaired spins.[16] The first level of excitation, the singlet form, is unstable either returning to a "ground state" or undergoing "spin inversion" to a triplet state.[16] In the case of HpD, approximately 90% of the energized photosensitizer ends up in the excited triplet state.[16]

Once the excited triplet HpD has been formed, there are two possible reactions which can occur. The first is a Type I reaction where excited HpD can react with either an organic substrate or with oxygen.[18,19] When it reacts with an organic substrate it results in the production of oxidized compounds.[18,19] When excited HpD reacts with oxygen, a superoxide radical ion (0_2^-) is produced which then reacts with organic substrates in the cell, producing

oxidized products.[18,19] This Type I reaction is dependent on the concentration of oxygen and the concentration of the substrates.[20] If the substrate availability or concentration is too low or if the oxygen concentration is too high, then the excited HpD will transfer its energy directly to oxygen resulting in singlet oxygen ($^1O_2$).[20,21] This then leads to a Type II reaction.[20,21]

Type II reaction begins with the transfer of energy from the excited triplet state HpD to ground state oxygen (which is already in a "triplet" state) producing excited singlet state oxygen.[20] Singlet state oxygen reacts with electron-rich organic substrates, resulting in the production of peroxides and other oxidized compounds.[21,22] The singlet oxygen can also react with cellular aminoacids such as histidine, tyrosine, tryptophan and cysteine.[21,22] When organic molecules in cell membranes are changed either by the oxidative process or into peroxides, it increases the fragility of all the cellular membranes.[16] When these membranes are structurally part of cellular organelles, this fragility leads to the organelles' destruction and thereby causes cell death.[16] When the cell wall is damaged from this reaction, it can predispose to cell lysis.[16]

The exact mechanism of PDT-induced cell cytotoxicity is still under debate. Some studies have suggested that the target site is the outer cell membrane.[23] It is thought that HpD binds to the cell membrane. Following the photochemical reaction, changes in the cell membrane lead to inhibition of normal transmembrane-transport mechanisms and eventually to cell death. Several researchers have suggested that the primary site of action is cellular organelles, specifically the mitochondria, and experimentally demonstrated a

significant decrease in intracellular ATP production following photodynamic therapy.[24,25] Cell cytotoxicity positively correlated with the decrease in cellular ATP production from the mitochondrion.[24,25]

Other research has demonstrated that the primary site of damage leading to tumor destruction is actually the tumor vasculature. HpD has been shown to be highly concentrated in the vascular stroma of tumors.[26] A subsequent study also demonstrated vascular collapse leading to subsequent cell death after PDT.[27] In a third study in which an *in-vivo* microscopic technique was used, bleaching of blood vessels and cessation of blood flow were observed and in their report, the authors concluded that cell kill and subsequent tumor destruction were secondary to microcirculatory collapse.[28]

A third site of action has been suggested.[29] Multiple experiments have demonstrated up to an 80% decrease in DNA synthesis and up to a 60% decrease in protein synthesis immediately following PDT.[20] These treatments stopped cell replication for a significant period of time but the cells eventually recovered and normal replication resumed. The researchers reporting on these experiments concluded that PDT did have a deleterious effect on DNA and protein synthesis but that damage to cells was reversible with time and cells were able to repair themselves and eventually resume replication.

It is very possible that all of these mechanisms could play significant roles in tumor response to photodynamic therapy.

What determines the accumulation of "active" components of hematoporphyrin derivative (HpD) in cells is still unknown. There have been many theories postulated. Recent studies have documented a porphyrin-lipoprotein affinity.[30] Binding sites for low density lipoproteins (LDL) exist on the cell membranes. The relative number of binding sites is different for various tissues and is elevated in neoplastic cells.[31] The affinity of neoplastic cells for HpD appears to correspond to the number of LDL binding sites.[32]

It has also been shown that HpD can also bind to plasma albumin.[33] It is apparent that HpD is taken into cells in two phases, an initial rapid phase and a second slower phase. It has been shown that uptake of HpD by cells is not proportional to free HpD concentration.[33] The same study demonstrated an enhanced uptake by an acidic microenvironment and by hyperthermia. These experiments do not suggest that HpD is preferentially taken up or retained by neoplastic cells. It seems that certain properties of neoplastic tissue must be responsible for the selective retention or concentration of HpD. Leaky vasculature, poor lymphatic drainage, increased uptake by macrophages, low pH and elevated concentration of lipoprotein receptors all factor into the tumor cells' affinity for HpD.[34]

Hypoxic Cytotoxic Adjuvants:

Hypoxic cells in solid tumors tend to be resistant to both radiation and chemotherapy.[35] Radiation affects tumor cells via two mechanisms.[36] The first mechanism is photo-energy which causes the release of a secondary electron able to directly damage cellular DNA. A second, more indirect mechanism is the reaction of a secondary electron with cellular water, producing the hydroxyl radical, which in turn leads to DNA damage. The chemical change in DNA is fixed in the presence of oxygen, preventing any form of cellular repair. This resistance of hypoxic cells to standard forms of therapy for cancer, especially radiotherapy, spurred interest in a group of compounds known as radiosensitizers. These compounds have also proven to be effective adjuvant therapy following PDT.[37]

Radiosensitizers mimic oxygen in that they can react with cellular components damaged by radiation, fix the radiation lesion and prevent cellular repair. These radiosensitizers are a group of electron affinic compounds, the most common being 2-nitroimidazoles.[38] The hypoxic-cytotoxic properties of these compounds positively correlate with their electron affinity despite the fact that the two mechanisms are unrelated.[39] Radiation sensitization occurs almost immediately since all that is required is a transfer of electrons, but hypoxic-cytotoxicity requires substantial exposure time to develop.[40] The cytotoxic property is thought to be due to the reductive metabolism of the drug resulting in the production of cytotoxic substances. The efficiency of the hypoxic cytotoxic adjuvant is thought to be cell-cycle dependent, with maximal lethal effect on

cells in early S-phase.[41] The efficiency of these drugs as radiosensitizers is not affected by temperature but their cytotoxic effect on hypoxic cells is potentiated by hyperthermia.[42]

The most common radiosensitizing agents, or hypoxic cytotoxic agents are 2-nitroimidazoles. These include misonidazole [1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol], also known as Ro-07-0582, and its antibiotic predecessor, metronidazole.[43] A newer agent RSU-1069 [1-(2-nitro-1-imidazolyl)-3-aziridino-2-propanol], is a similar cyclic-structured compound, but the addition of an aziridine ring, a monofunctional alkylator, increases its cytotoxicity.[44] The newest hypoxic cytotoxic agent is SR-4233 [3-amino-1,2,4-benzotriazine 1,4 dioxide]. Structurally, it is totally dissimilar to the 2-nitroimidazole compounds and it has completely different pharmacokinetics.[45] If the cytotoxicity of misonidazole is given a relative value of approximately 1.0, then the cytotoxicity of metronidazole and RSU-1069 are approximately 0.15 and approximately 100-200 respectively. SR-4233 is still far more cytotoxic with a relative cytotoxicity approximately 250-350 times greater than misonidazole.[45]

Nuclear Magnetic Resonance (NMR) Spectroscopy:

The technology of nuclear magnetic resonance (NMR) imaging and spectroscopy provide an ideal method of chemical analysis *in-vivo* without disturbing the biological system under investigation. Specifically, NMR allows assessment of cellular metabolism in neoplastic cells after the application of various therapeutic modalities. NMR spectroscopy has been used in the

chemical analysis of HpD.[9] More recently, ^{31}P NMR spectroscopy has been used for *in-vivo* analysis of tumor metabolism after treatment with PDT.[46,47]

The theory behind nuclear magnetic resonance imagery and spectroscopy is explained by Bradley.[48] Nuclei of atoms in molecules align themselves in the large magnetic field of the NMR machine. A second magnetic field is applied as an electro-magnetic radio frequency pulse and this changes the alignment of the nuclei for as long as the pulse lasts. When this second electro-magnetic field is gone, the nuclei eventually return to their original alignment under the influence of the large magnetic field produced by the NMR unit. As nuclei re-align themselves, they emit energy in the form of radiowaves which can be detected, measured and recorded. The emission of this energy is termed "relaxation". Two components of relaxation are measurable and unique to those nuclei with which share the same surrounding electron configuration.

The "longitudinal relaxation time" or "spin lattice relaxation time" (T_1) is the time it takes these nuclei to initially become polarized in the fixed magnetic field of the NMR unit, and T_1 is also the time it takes for the nuclei to become re-aligned, in the fixed magnetic field, after having their alignment altered by the electromagnetic radio frequency pulse. The "transverse magnetic relaxation time" or "spin-spin relaxation time" (T_2) is a measure of how long nuclei hold their altered orientation after removal of the electro-magnetic radiofrequency pulse, before returning to their original orientation under the influence of the fixed magnetic field of the NMR unit. The "longitudinal relaxation time" or "spin lattice relaxation time", T_1, is characteristically long in solids, shorter in

liquids and shortest in gases, while the "transverse magnetic relaxation time" or "spin-spin relaxation time", T_2, is long in gases, shorter in liquids and shortest in solids.

The pulsed electromagnetic radiofrequency field is, in the case of NMR spectroscopy, emitted from a solenoidal coil placed on the area to be "scanned". A current through the coil induces an electromagnetic field which excites or alters the orientation of the involved nuclei. When the current is interrupted the coil acts as a receiver for the resonance radiowave signals emitted from the nuclei as they re-align in the fixed magnetic field of the NMR unit.

Different molecules with the same nuclear configuation will respond with different and unique nuclear resonance depending on the electron configuration around the nucleus. The electrons shield the nucleus. Thus each nucleus with a unique electron configuration will have an individual, unique resonance.

Each peak on an NMR spectrum represents nuclei with the same chemical (or electrical) environment. The area under this peak, is directly proportional to the number of nuclei present. In this way it is possible to get accurate relative quantification of chemical species existing in the sample scanned. An accurate, absolute quantification is not possible with present technology.[49]

There are three nuclei which are commonly used in NMR spectroscopy: Hydrogen (H), Carbon^{13} (^{13}C), Phosphorus^{31} (^{31}P). When doing NMR

spectroscopy for analysis of cellular metabolism, especially cellular energetic, ^{31}P is an ideal nucleus to scan. Phosphorus plays a central role in cellular metabolism, is present in large concentrations and is ubiquitous throughout the cell. Signals from phospholipids, bone and other solid sources of phosphorus do not interfere with ^{31}P NMR spectroscopy. Phosphorus also lacks some of the electromagnetic spin complications encountered when scanning for H or ^{13}C resonance.[49] For all of the above reasons ^{31}P NMR spectroscopy is more suited for *in-vivo* assessment of tumor metabolism and hypoxia.

^{31}P NMR spectroscopy has been used *in-vivo* to detect the metabolic response of tumors to various forms of therapy. Adenosine triphosphate (ATP) levels in cells, which were detected by ^{31}P NMR spectroscopy, were significantly reduced after HpD sensitized PDT.[50] The decreased ATP levels correlated positively with decreased cell survival. These findings suggest that PDT interfered with mitochondrial production of ATP, leading to lethal disruption of cellular metabolism.[50] Research with ^{60}Co-therapy monitored by ^{31}P-NMR spectroscopy demonstrated a decrease in ATP levels with concomitant increases in inorganic phosphorus and di-phosphate esters.[51] Ng, et al, documented an increase in intracellular pH.[51] Subsequent work with PDT monitored by ^{31}P-NMR spectroscopy has also shown a significant decrease in ATP levels and increases in inorganic phosphorus and diphosphate esters.[47]

Hypothesis:

Previous unpublished data from the Cross Cancer Institute, Edmonton, was able to demonstrate PDT induced hypoxia, in both Dunning tumor models, using a sensitizer adduct technique for measuring tumor hypoxia. This technique delineated an increased hypoxic fraction of tumor cells as the light dose administered during PDT was increased. Despite the accuracy and sensitivity of the sensitizer adduct technique for measuring tumor hypoxia, it is an *in-vitro* test. Metabolic changes in tumors after therapy have been detected using ^{31}P NMR spectroscopy and similar changes have also been seen with variations in tissue oxygenation.[52,53] Several researchers have used ^{31}P NMR spectroscopy to monitor changes, *in-vivo*, in tumors after photodynamic therapy.[47,54] A study was therefore undertaken to carefully induce graded degrees of hypoxia in the Dunning tumor model using PDT and to attempt to detect and quantitate these changes using *in-vivo* ^{31}P NMR spectroscopy.

In contrast to most histologically-normal tissue (with the exception of the reticulo-endothelial system and the kidneys), neoplastic tissue selectively retains HpD.[34,55] Therefore, in order to maximize normal tissue sparing both clinical and experimental protocols have called for the administration of photosensitizer 24-72 hours prior to PDT.[32,55,56] With interstitially applied PDT, the limiting factor in tissue damage is not as dependent on tissue concentration of the photosensitizer as it is on photodosimetry of the light energy within the tissue.[57,58] Since maximal tissue concentration of photosensitizer occurs 4-6 hours after systemic administration, interstitially applied PDT may be more

effective if photoirradiation occurs within this time frame.[32,37] A study was therefore undertaken to measure delay in tumor regrowth in the Dunning -AT tumor, comparing the biological effects of interstitially applied PDT 4 hours after administration of Photofrin II, versus PDT 24 hours after administration of Photofrin II.

The cytotoxicity of 2-nitroimidazole compounds, Misonidazole (MISO) and RSU-1069, to hypoxic cells has been well documented.[43,44] The *in-vitro* cytotoxicity of the benzotriazine, SR-4233, to hypoxic cells has also been proven.[45] RSU-1069 and SR-4233 are significantly more cytotoxic to hypoxic cells than MISO.[45] In addition the *in-vivo* effect of combination PDT and MISO was shown to significantly prolong the delay in tumor regrowth and local control in the Dunning tumor model.[37] This prompted a comparative study of tumor growth delay in the Dunning -AT tumor after combination PDT and hypoxic cytotoxic therapy using either MISO, RSU-1069 or SR-4233.

The ^{31}P NMR spectroscopy of Dunning tumors sensitized by Healux H628, before and after PDT, prompted an April 1988 comparison of Healux H628 and Photofrin II. This comparison included an assessment by ^{31}P NMR spectroscopy, before and after PDT (2000 J/cm3), of Dunning -AT tumors sensitized by either Healux H628 or Photofrin II. In addition, the biological effect of combination therapy with MISO and PDT (1000 J/cm^3), sensitized by Healux H628, was compared to combination therapy with MISO and PDT (1000

J/cm^3), sensitized by Photofrin II. This comparison was accomplished by contrasting the delay in regrowth of Dunning -AT tumors in a control and two treatment groups.

MATERIALS AND METHODS:

Animal Model:

All animal experiments were performed in the Radiobiology Animal Laboratory and the Department of Surgery Laser Laboratory at The Cross Cancer Institute, Edmonton. F_1 hybrid male rats, from Fischer (female) x Copenhagen (male) matings, were obtained from breeding stock of the Health Sciences Laboratory, Animal Services, University of Alberta, Edmonton. They were housed before tumor implantation with a maximum of five animals per cage and were fed rodent chow and water as desired. After tumor implantation and after the tumors became palpable, the rats were housed in individual cages. The animals were at least six weeks post weaning before tumor implantation. Once the tumors were palpable and the animals housed in individual cages, they were randomly selected for the various experimental protocols.

Tumor Model:

In 1963 Dr. W. F. Dunning described and successfully transplanted a spontaneous tumor of the rat prostate.[59] The tumor is now called the Dunning R3327 -H and closely resembles a well-differentiated, human prostatic adenocarcinoma.[60] Subsequent variants of the initial tumor were identified showing different characteristics in terms of growth rate, degree of anaplasia,

hormone sensitivity, vascularity and hypoxic cell fraction.[60] The tumor initially identified by Dunning was relatively slow growing with a doubling time of 15-20 days.[61,62] This tumor has since been designated the Dunning R3327 -H tumor and is hormone sensitive and well vascularized.[61,62]

In 1976 Smolev and Lubaroff separated out an anaplastic cell line from the original Dunning tumor. It has subsequently been designated the Dunning -AT tumor. This tumor was much faster growing with a doubling time of two days. It was insensitive to hormonal manipulation and was relatively avascular with a large fraction of hypoxic cells.[59,61]

The Dunning R3327 -H tumor was obtained from the Papanicalaou Institute, Miami, Florida, in 1979. Since that time the tumor has been maintained in successive F_1 hybrid male rat generations. Tumor histology is checked with every transfer to ensure that no tumor drift has occurred. The Dunning R3327 -AT tumor was obtained from the James Buchanan Brady Urologic Institute, Johns Hopkins Hospital, Baltimore, Maryland, in 1983. It has also been maintained in successive F_1 hybrid generations. As was the case with the Dunning -H tumor, the histology of the -AT tumor has been monitored with each successive tumor transfer.

Animal Anesthetic Technique:

The rats were anesthetized with halothane gas for tumor measurement and tumor transfer and with intraperitoneal (i.p.) injection of Ketamine and Rompon for all the experimental procedures. Ketamine was administered at 75 mg/kg body wt and Rompon was administered at 7.5 mg/kg body wt.

Tumor Transfer Technique:

Tumors selected for transplantation were sugically harvested from donor animals and maintained on ice in Hanks Balanced Salt Solution until ready for implantation. The tumors were sectioned into 1-2 mm cubes and readied for implantation into subcutaneous tissue of the flank, in Fischer x Copenhagen F_1 hybrid male rats. A small (less than 1 cm) incision was made in the flanks of the recipient rats, the tumor placed subcutaneously and the incision closed with a surgical skin clip. The skin clips were removed 7-10 days after inplantation. A latency period of 10-14 days was observed before the -AT tumors became palpable. The latency period for the -H tumor was significantly longer (approximately 8-12 weeks) before it became palpable.

Tumor Measurement:

The technique used in these experiments for tumor measurement has been previously described.[63] With the rats under halothane anesthesia the tumors were measured twice weekly with calipers in three mutually orthogonal dimensions. Tumor volumes were computed using the formula for the volume of an ellipsoidal mass, $Vol = \pi/6 * D1 * D2 * D3$. From each caliper measurement, 1.3 mm was subtracted, which represents the average thickness of a double layer of rat skin.

Photosensitizers:

Until July 1987, Photofrin II (Photomedica Inc., Raritan, NJ.) had been freely available, gratis, for experimental protocols. However, because of a change in ownership of the company, Photofrin II was unobtainable between August 1987 and April 1988. After April 1988 Photofrin II (Quadra Logic Inc., Vancouver B.C.) was again provided gratis for PDT experimental protocols. Photofrin II contained dihematoporphyrin ether, in isotonic saline, 2.5 mg/ml. In the experimental protocol comparing delay in tumor regrowth after PDT, either 4 hours or 24 hours after the administration of photosensitizer and the experimental protocols comparing the photosensitizing effectiveness of Photofrin II and Healux H628, Photofrin II was administered i.p., 15 mg/kg body wt.

Since Photofrin II was unavailable from August 1987 to April 1988, a substitute photosensitizer was needed so that PDT research at the Cross Cancer Institute could continue uninterrupted. J.J. Lai and B.W. McCaul, et al, presented results using a new photosensitizer, Healux H628, at The Clayton Foundation Conference on Photodynamic Therapy in Los Angeles, 1987.[64] The results of their experiments clearly demonstrated that Healux H628 was at least as effective, if not more effective than Photofrin II, *in-vitro* and *in-vivo*. In addition to this, they had shown that the Healux product was more stable in storage than Photofrin II.[64] On the basis of these studies, Healux H628 (Healux Inc., Sunnyvale, California) was chosen as the substitute photosensitizer for our studies. It contained polyhematoporphyrin esters, 2.4 mg/ml and was administered i.p., 15 mg/kg body wt., when used as a photosensitizer.

With the renewed availability of Photofrin II in April, 1988, comparative studies were carried out testing the respective phototoxicity of both Healux H628 and Photofrin II. The first of these studies utilized ^{31}P NMR spectroscopy to assess the degree of hypoxia induced in Dunning -AT tumors by PDT with either Healux H628 or Photofrin II as photosensitizers. The second study compared delays in tumor regrowth in the Dunning -AT tumor after combination therapy with Misonidazole (MISO) and PDT sensitized with either Healux H628 or Photofrin II.

Laser Phototherapy:

The light source used in all the PDT studies was a Coherent CR-599 Argon-driven dye laser tuned to 630 nm. A beam-splitting device supplied light equally to four, fused silica optical fibers, with a maximal total output of 1.5-2 watts. The tips of the fibers had been modified according to the technique described by Arnfield, et al.[65] These cylindrical irradiator tips diffused light laterally with equal intensity over a distance of 1 cm. A fixed plexiglass grid was used for fiber placement. Translucent plastic needles were inserted through the grid, into the tumor, 0.94 cm apart. The optical fibers were then inserted into the translucent needles. The power output from the fibers varied, depending on the experiment, from 7.5 mW/fiber to 150 mW/fiber. Central tumor temperature was monitored with a fine needle thermistor and was always kept below 41 °C by surface alcohol cooling.

Specific Experimental Protocols:

A. PDT Induced Metabolic Changes in the Dunning -H and -AT Tumors, Detected by 31P NMR Spectroscopy:

^{31}P NMR spectroscopy was used to detect changes in phosphorous metabolism as a result of photodynamic induced hypoxia, at various times after treatment and at various light energy doses. Each animal acted as its own control.

Day #0: Control ^{31}P NMR spectroscopy

Day #1: 4 hr prior to PDT 15 mg/kg Healux H628, i.p.

PDT (light dose 100-2000 J/cm^3, 7.5-150 mW/fiber)

1 hr post-PDT, repeat ^{31}P NMR spectroscopy

^{31}P NMR spectroscopy was carried out in the Small Bore Experimental NMR Lab at the Walter C. MacKenzie Health Sciences Center, Edmonton. The resulting ^{31}P NMR spectra resonance peaks corresponded to phosphorous monoesters (PME), inorganic phosphorous (Pi), phosphorous diesters (PDE), phosphocreatine (PCr), Gamma ATP, Alpha ATP and Beta ATP, and were obtained at 2.35 Tesla (T) using a 40 cm bore magnet, 1.5 cm electromagnetic coil and a Brucker CXP spectrometer. Five hundred and twelve scans were obtained for each sample and signals were corrected for relaxation time differences. The shift of the Pi peak as determined by the formula, δPi = Pi-PCr, was then used in the formula, pH = 6.77 + log(δPi-3.29/5.68-δPi), to determine the pH of the tumors. In order to determine the relative amounts of each chemical component of the phosphorus spectra, the areas under the curve were derived for each resonance peak by using computer equipment and a special software program. The area values were standardized and then the means and standard deviations were calculated for the 25 -AT control spectra and the 28 -H control spectra.

For the Dunning -AT tumor ^{31}P NMR spectroscopy was obtained before and after PDT at 100 J/cm^3 in 4 animals, PDT at 500 J/cm^3 in 8 animals, PDT at 1000 J/cm^3 in 7 animals and PDT at 2000 J/cm^3 in 6 animals. For the Dunning -H tumor, ^{31}P NMR spectroscopy was obtained before and after PDT

at 100 J/cm^3 in 5 animals, PDT at 500 J/cm^3 in 8 animals, PDT at 1000 J/cm^3 in 5 animals, PDT at 1500 J/cm^3 in 4 animals and PDT at 2000 J/cm^3 in 6 animals.

The areas under the resonance peaks were summed, normalized and the means and standard deviations computed. The relative percentage of the total phosphorous signal for each of the resonance peaks was then compared for each of the light doses, to determine if the spectra changed significantly with increasing light doses. The pH was derived from the shift of the Pi resonance peak.

B. Comparison of Delay in Tumor Regrowth Following PDT after Photosensitizer Administration 4 hours prior to PDT vs 24 hours Prior to PDT:

In this second experiment anaplastic -AT tumors were exclusively used. Animals were implanted as previously described in this text and were randomly selected for the experimental groups when they reached 750-1050 mm^3. There were 3 experimental groups. The first group was a control and received no drugs or therapy. Tumors in this group of animals were measured bi-weekly until they reached 10x their initial measured volume. The second group of animals received 15 mg/kg Photofrin II, i.p., 4 hours prior to PDT (2000 J/cm^3). The tumors were then followed with bi-weekly volume determinations until they reached more than 10x their treatment volume. The third experimental group received 15 mg/kg Photofrin II, i.p., 24 hours prior to PDT (2000 J/cm^3). The tumors were also followed with bi-weekly measurements until they grew to greater than 10x their treatment volume. All the photodynamic therapy was performed under Ketamine/Rompon anesthesia and all tumor measurements

were performed under light halothane anesthesia as previously described in this text. Growth delay curves for each of the three experimental groups were plotted and compared.

C. Comparison of Combination Photodynamic and Hypoxic Cytotoxic Chemotherapy with Misonidazole, RSU-1069 and SR-4233:

In this third experiment only the Dunning -AT tumor was used. There were four experimental groups. The first group (n=10) was a control group which received no drugs or therapy. The second group (n=9) received Healux H628, 15 mg/kg body wt, 4 hours prior to PDT and 0.5 mg/g MISO, dissolved in sterile isotonic saline (30 mg/ml), 30 min prior to PDT. The third group (n=8) received Healux H628,15 mg/kg body wt, 4 hours prior to PDT and 0.05 mg/g RSU-1069, dissolved in 5-10% DMSO (3 mg/ml), 30 min prior to PDT. The fourth group (n=5) received Healux H628, 15 mg/kg, 4 hours prior to PDT and 0.03 mg/g SR-4233, dissolved in 5-10% DMSO (2 mg/kg), 30 min prior to PDT. All three hypoxic cytotoxic agents were administered at 1/3 their respective LD_{50} dose.[39,45] In all three treatment groups, the photodynamic irradiation was delivered by four fiber-optic cylindrical irradiators, previously described, 150 mW/fiber, over one hour, for a total dose of 1000 J/cm^3. A lower light dose would be less lethal or curative but would still induce enough tumor hypoxia to allow the hypoxic cytotoxic agents to be effective. This would hopefully increase the proportional effect on the delay of tumor regrowth that was directly the result of the cytotoxicity from adjuvant agents rather than phototoxicity from the PDT. Again, all experimental groups were followed with bi-weekly tumor volume measurements. The untreated controls were followed until they grew to

> 10x their first measured volume. All three treatment groups were followed until they reached > 10x their treatment volume.

D. Comparison of Healux H628 and Photofrin II:

In our attempt to compare the effectiveness of Healux H628 and Photofrin II as photosensitizers, we randomly selected a total of 10 animals, with Dunning -AT tumors, and entered them into the same experimental protocol as already described in the ^{31}P NMR spectroscopy section. The only variable was the photosensitizer. Four animals were sensitized with Photofrin II, 15 mg/kg body wt, 4 hours prior to PDT (2000 J/cm^3). These animals acted as their own controls and underwent ^{31}P NMR spectroscopy 24 hours prior to and 1 hour after PDT. Their control and post-PDT ^{31}P NMR spectra were compared to the spectra of 6 animals, also with Dunning -AT tumors, who were sensitized with Healux H628, 15 mg/kg body wt., instead of Photofrin II. The ^{31}P NMR spectroscopy was performed and analyzed in the same manner as described previously in this thesis.

In a further attempt to compare the biological effectiveness of Healux H628 to Photofrin II, another experimental group of F_1 hybrid animals with implanted Dunning -AT tumors were randomly selected. This experimental group (n=4) received Photofrin II, 15 mg/kg body wt, 4 hours prior to PDT (1000 J/cm^3) and 0,5 mg/g MISO, dissolved in sterile isotonic saline (30 mg/ml), 30 minutes prior to PDT. As with the previous treatment groups, tumor

regrowth was followed until the tumors reached >10x their treatment volume. These results were then compared to a similarly treated group of F_1 hybrid male rats, with Dunning -AT tumors (n=9), which were treated by combination MISO and PDT sensitized by Healux H628.

RESULTS:

A. PDT Induced Hypoxic Changes in the Dunning -H and -AT Tumors as Measured by ^{31}P NMR spectroscopy.

The present study was undertaken to determine if *in-vivo* ^{31}P NMR spectroscopy was capable of detecting and quantifying PDT induced tumor hypoxia. Experimental protocol and method of analysis used to interpret data has already been reviewed in this thesis.

The characterization of untreated Dunning -H tumors by ^{31}P NMR spectroscopy is necessary in order that a comparison can be made with tumors treated by PDT. In addition, a comparison with ^{31}P NMR spectroscopy of untreated Dunning -AT tumors may demonstrate significant spectroscopic differences between the two tumors which might, in turn, represent differences in tumor biology. Table 1 displays the relative amounts of each spectral peak in control ^{31}P NMR spectra for 28 Dunning -H tumors. Table 2, shows the same data but lists the tumors in order of increasing volume.

A comparison was made between Dunning -H tumors less than 1500 mm^3 (n=7) and tumors greater than 3000 mm^3 (n=5). This data is presented in Table 3. The mean and standard deviation for tumor volume, PME, Pi, PDE, PCr, gamma, alpha, and beta ATP are listed. There was a statistically significant difference between the mean tumor volume for each group (analysis of variance, Bonferroni t-test, $P<0.001$). This is, however, the only significant difference

between the two groups. There was no significant difference between the mean values for PME, Pi, PDE, PCr, gamma, alpha and beta ATP.

Table 4 presents the results of ^{31}P NMR spectroscopy after PDT at various light doses (100, 500, 1000, 1500, and 2000 J/cm^3) for the Dunning -H tumor (n=28). Means and standard deviations for each group treated by PDT at a specific light dose were calculated for volume and the relative amounts of each spectral peak. A comparison was made with untreated control values and between values derived at each light dose (100, 500, 1000, 1500, and 2000 J/cm^3) used during PDT. The only statistically significant difference noted was an increase in the relative amount of Pi in the group of tumors treated by PDT at 100 J/cm^3 total light dose (analysis of variance, Bonferroni t-test, $P<0.05$). There were no other significant changes in ^{31}P NMR spectra of tumors at any other light energy level used for PDT.

The characterization of untreated Dunning -AT tumors by ^{31}P NMR spectroscopy is necessary in order that a comparison can be made with tumors treated by PDT. In addition, a comparison with ^{31}P NMR spectroscopy of untreated Dunning -H tumors may demonstrate significant spectroscopic differences between the two tumors which might, in turn, represent differences in tumor biology. Control ^{31}P NMR spectra on 25 Dunning -AT tumors is presented in Table 5 and Table 6. Table 6 lists the tumors in order of increasing volume.

A comparison was also carried out between Dunning -AT tumors of different sizes. The data comparing Dunning -AT tumors less than 1500 mm^3 (n=6) and those greater than 3000 mm^3 (n=5) is presented in Table 7. Again there is a statistically significant difference between the mean tumor volume of the small and large groups (analysis of variance, Bonferroni t-test, $P<0.001$). However, when comparing the ^{31}P NMR spectra for small and large -AT tumors there is no significant difference between them.

The relative amounts of phosphorus moeties measured by ^{31}P NMR spectroscopy in the Dunning -AT tumors (n=25) after PDT at 100, 500, 1000, and 2000 J/cm^3 total light dose, is presented in Table 8. A comparison was made with untreated control values and between the values derived at each light dose (100, 500, 1000, and 2000 J/cm^3) used during PDT. No statistically significant changes in the spectra were identified. Specifically, no statistically significant changes were documented as the light energy level used during PDT was increased.

The biological and histological differences between the Dunning -H and -AT tumors have already been discussed. The well perfused and therefore well oxygenated Dunning -H tumor has a lower proportion of hypoxic cells than the avascular and poorly oxygenated Dunning -AT tumor. A comparison between 28 Dunning -H control ^{31}P NMR spectra and 25 Dunning -AT control ^{31}P NMR spectra is made in Table 9. The mean tumor volume of each control group was not significantly different (*analysis of variance, Bonferroni t-test*, $P=0.340$). There were, however, significant differences between the ^{31}P NMR

spectra of the two tumor groups. In the Dunning -AT tumors, the relative amounts of Pi were increased while the relative amounts of PCr and PDE were decreased when compared with the Dunning -H tumors (analysis of variance, Bonferroni t-test, $P<0.05$).

B. Comparison of PDT Induced Growth Delay in the Dunning -AT Tumor, 4 hour after Sensitizer Administration *vs* 24 hour after Sensitizer Administration:

When laser light for PDT is delivered through interstitially placed fiber optics, normal tissue sparing is accomplished by the intratumor placement of the fiber optic illuminator tips. This intratumor placement minimizes normal tissue photoirradiation because the light dose falls off rapidly within a short distance from the illuminator tips.[57] It is unknown if changing the temporal relationship between the photosensitizer administration and photoirradiation effects tumor response to interstitially applied PDT.

Photofrin II, 15 mg/kg body wt., was administered to 3 groups of F_1 hybrid male rats with Dunning -AT tumors implanted in their flanks. In the group ($n=10$) in which Photofrin II was administered 24 hours prior to PDT, there was one cure (no palpable tumor at 30 days follow up) and the mean (± standard deviation) time to regrwoth to >10x treatment volume for the remaining tumors was 19.56 ± 4.33 days. In the group ($n=11$) in which Photofrin II was administered 4 hours prior to PDT, there were three cures (no palpable tumor at 30 days follow up) and the mean (± standard deviation) time to regrowth to >10x treatment volume for the remaining tumors was 21.75 ± 4.33 days. The cure rates between these two treatment groups were not statistically

different (analysis of variance, Bonferroni t-test, $P>0.05$). The regrowth rate of the remaining tumors in both groups was not statistically different (analysis of variance, Bonferroni t-test, $P>0.05$). However when these two treatment groups were compared to a third or control group ($n=18$) there was a statistically significant difference between the mean (± standard deviation) time to regrowth to >10x treatment volume of 16.06 3.03 days for the control group (analysis of variance, Bonferroni t-test, $P<0.01$). These results are graphically illustrated in Figure 1.

C. Delay of Tumor Regrowth after Combination Photodynamic and Hypoxic Cytotoxic Therapy using Misonidazole, RSU-1069 and SR-4233.

Hypoxic radiosensitizers are selectively cytotoxic to hypoxic cells, and potentiate the tumor response to PDT.[27,37,66,67,68,69] An experiment was designed to compare the biological effects of MISO, RSU-1069, SR-4233 when used in combination with photodynamic therapy (PDT). The newer agents are more cytotoxic *in-vitro*, when compared to MISO.[45]

Treatment with PDT and MISO combined resulted in a mean (± standard deviation) time to regrowth to >10x treatment volume of 20.56 ± 6.60 days. By comparison untreated controls took 13.6 ± 1.96 days (mean ± standard deviation) to regrowth to >10x their initial volume. This difference in delay of tumor regrowth was significant as tested by analysis of variance, Bonferroni t-test, $P<0.01$. The group which was treated with combination PDT and RSU-1069 also produced significant delays in tumor regrowth with a mean (± standard deviation) time to regrowth to >10x treatment volume of 22.38 ± 3.29

days. This delay did not differ significantly from the MISO group. The group treated with combination PDT and SR-4233 also showed a significant (analysis of variance, Bonferroni t-test, $P<0.01$) delay in tumor regrowth compared to controls with a mean (± standard deviation) time to regrowth to > 10x treatment volume of 20.4 ± 2.07 days. There was no significant statistical difference in the delay of tumor growth between the three hypoxic cytotoxic agents used in combination with PDT (Figure 2). The experimental group which received SR-4233 in combination with PDT was initially composed of 8 animals but 3 died during follow-up before their tumors had regrown, presumably because of the systemic toxicity of SR-4233. No other animals in any of the other groups died during follow-up.

D. Comparison of Photosensitizers, Healux H628 and Photofrin II by PDT Induced Changes in ^{31}P NMR spectroscopy and by Delay of Tumor Regrowth after combination MISO and PDT.

^{31}P NMR spectroscopy has been used to detect PDT induced tumor hypoxia *in-vivo*.[70,71] The sensitizer adduct technique has also been able to detect PDT induced tumor hypoxia *in-vitro* (unpublished data from the Cross Cancer Institute, Edmonton). In both of these cases Photofrin II or its predecessor Photofrin I were used as photosensitizing agents. As noted above, ^{31}P NMR spectroscopy was unable to detect any changes in Dunning tumors before and after Healux H628 sensitized PDT. These findings differed from those of other researchers who had detected PDT induced cellular changes with ^{31}P NMR spectroscopy.[54,71] This prompted a comparison of Healux H628 and Photofrin II when Photofrin II became available in the Spring of 1988.

Two groups of F_1 hybrid male rats with Dunning -AT tumors implanted in their flanks were used in this experiment. Tumors acted as their own controls and ^{31}P NMR spectroscopy was done 24 hours prior to treatment with PDT sensitized with either Photofrin II or Healux H628. In the post-PDT ^{31}P NMR spectra of tumors treated by Photofrin II photosensitized PDT ($n=4$), there was a statistically significant increase in the percentage that Pi contributed to the total phosphorus signal (analysis of variance, Bonferroni t-test, $P<0.01$), when compared to the groups control ^{31}P NMR spectra. In addition, when compared with control ^{31}P NMR spectra, there was a statistically significant decrease in the percentage that the three ATP and PDE moeties contributed to the total phosphorus signal (analysis of variance, Bonferroni t-test, $P<0.01$). The post-PDT spectra taken from the Healux H628 photosensitized tumors ($n=6$) were not significantly different from their control, pre- PDT spectra. The results of control ^{31}P NMR spectroscopy for both groups are presented in Table 10. The mean (± standard deviation) tumor volumes in each of the experimental groups were not significantly different (Table 10). There were no statistically significant differences between the two groups control ^{31}P NMR spectra (Table 10). Table 11 displays the post-treatment ^{31}P NMR spectra for both the group treated by Healux H628 sensitized PDT and the group treated by Photofrin II sensitized PDT. A comparison of the two groups treatment ^{31}P NMR spectra is graphically illustrated in Figure 3.

Results comparing Healux H628 and Photofrin II using ^{31}P NMR spectroscopy of PDT treated Dunning -AT tumors prompted a second

experiment. A statistically significant delay in Dunning -AT tumor regrowth, after Photofrin II photosensitized PDT, has been previously demonstrated.[37,68] Therefore, an experimental protocol was established comparing tumor response to combination MISO plus PDT photosensitized by either Healux H628 or Photofrin II. The untreated control group consisted of 10 Dunning -AT tumors implanted in the flanks of F_1 hybrid male rats. The group treated by combination MISO and Healux H628 photosensitized PDT consisted of 9 Dunning -AT tumors implanted in the flanks of F_1 hybrid male rats. Four Dunning -AT tumors implanted in F_1 hybrid male rats, made up the Photofrin II arm of the protocol.

The results are illustrated graphically in Figure 4. Both treatments significantly prolonged the time to regrowth (analysis of variance, Bonferroni t-test, $P<0.01$), when compared to controls. The mean (± standard deviation) time to regrowth for the control group was 13.6 ± 1.96 days. The mean (± standard deviation) time to regrowth for the combination MISO and Healux H628 photosensitized PDT group was 20.0 ± 6.6 days. This delay was significantly shorter than the delay in regrowth seen after combination treatment with MISO plus Photofrin II photosensitized PDT (analysis of variance, Bonferroni t-test, $P<0.01$). The mean (± standard deviation) time to regrowth to >10x treatment volume for this last experimental group was 29.75 ± 8.8 days.

DISCUSSION:

As yet the characterization of untreated Dunning -H and -AT tumors by ^{31}P NMR spectroscopy has not been described, nor have the ^{31}P NMR spectra of these tumors been compared. These two rat prostatic carcinomas differ significantly in respect to their biological behavior and to the degree of cellular oxygenation.[60,61,63] The response of the Dunning -H and -AT tumors to therapy is also significantly different.[37,63] Control ^{31}P NMR spectra from 28 Dunning -H tumors and 25 Dunning -AT tumors is seen in Table 9. The comparison in Table 9 shows a significant difference between these two tumors as detected by ^{31}P NMR spectroscopy, *in-vivo*. Tumor volume may impact on the result of ^{31}P NMR spectroscopy.[72] As seen in Table 9, the mean (± standard deviation) tumor volume for each tumor group was not significantly different. Therefore, differences between the ^{31}P NMR spectroscopy of the Dunning -H and -AT tumors are not due to differences in tumor volume. ^{31}P NMR spectroscopy of tumors with different hypoxic cell fractions, should detect significantly different relative amounts of PME, Pi, PDE, PCr, gamma, alpha and beta ATP.[73] As can be seen in Table 9, the relative amount of Pi detected by ^{31}P NMR spectroscopy in the 25 control Dunning -AT tumors was statistically significantly greater (analysis of variance, Bonferroni t-test, $P<0.05$) than the relative amount of Pi measured in the 28 Dunning -H tumors. In addition, the relative amount of PCr detected by ^{31}P NMR spectroscopy in the 25 control Dunning -AT tumors was statistically significantly less (analysis of variance, Bonferroni t-test, $P<0.05$) than the relative amount of PCr measured in the 28 Dunning -H tumors. However, the relative amounts of ATP detected

by ^{31}P NMR spectroscopy in either the Dunning -H or -AT tumor was not significantly different. There was a statistically significant decrease in the relative amount of PDE measured by ^{31}P NMR spectroscopy in the Dunning -AT tumors compared to the ^{31}P NMR spectroscopy of the Dunning -H tumors. Irving, et. al., evaluated the ability of ^{31}P NMR surface coil spectroscopy to measure alterations in tumor metabolism with growth or after therapy.[74] These authors attributed decreases in PCr and corresponding increases in Pi and PDE to increased tissue acidity secondary to tumor hypoxia and anaerobic glycolysis.[74] They noted that these alterations in the relative amounts of PCr, Pi and PDE were present when comparing a well differentiated tumor to a more anaplastic tumor.[74] In addition, they documented the above noted alterations in ^{31}P NMR spectroscopy after tumor growth.[74] The difference between the relative amounts of PDE detected in the Dunning tumors might represent structural differences between tumor lines rather than physiological differences. PDE might be present in higher quantities in organized tissue such as muscle, and might therefore be more abundant in the histologically well differentiated Dunning -H tumor. PDE might simply contribute a smaller proportion to the chemical composition of the histologically, very poorly differentiated, Dunning -AT tumor. The differences in relative amounts of PDE detected by ^{31}P NMR spectroscopy might be secondary to differences in tumor chemical composition rather than differences in tumor physiology.

As tumor cells replicate and the tumor volume increases, the tumors' blood supply can become inadequate.[53,72] This biological process would imply that smaller tumors are inherently better oxygenated and better vascularized

than larger tumors of the same histologic type.[53,72] These changes in tumor oxygenation should be detectable by *in-vivo* ^{31}P NMR spectroscopy. A comparison between control ^{31}P NMR spectra of small and large Dunning -H tumors is made in Table 3. There is a statistically significant difference (analysis of variance, Bonferroni t-test, $P<0.001$) in the mean tumor volume between the small (less than 1500 mm^3) and the large (greater than 3000 mm^3) Dunning -H tumor groups. There were no other significant differences between the ^{31}P NMR spectroscopy of these two differently sized groups of Dunning -H tumors. A similar comparison between control ^{31}P NMR spectra of small and large Dunning -AT tumors is made in Table 7. There is a statistically significant difference (analysis of variance, Bonferroni t-test, $P<0.001$) in the mean tumor volume between the small (less than 1500 mm^3) and the large (greater than 3000 mm^3) Dunning -AT tumor groups. Again, this difference in tumor volume was the only significant difference noted between the small and large volume Dunning -AT groups.

The lack of difference in ^{31}P NMR spectra between small and large tumors was apparent in both the Dunning -AT and -H tumor lines. These findings could have several plausible explanations. It is possible that ^{31}P NMR spectroscopy is simply incapable of detecting differences in cellular metabolism that were present between the small and large Dunning -H and -AT tumors. As advanced as we might perceive medical research technology to be, there are limitations associated with the technology of ^{31}P NMR spectroscopy. These deficiencies are present in both the hardware and the software used in ^{31}P NMR spectroscopy. The inability to measure the absolute amount of phosphorus

molecules present in a scanned sample is an example of these limitations. The technology today limits us to relative measurements of phosphorus molecules.

A second and possibly more significant explanation for the lack of differentiation by ^{31}P NMR spectroscopy between small and large Dunning tumors, may be related to the particular sample of tumor scanned by the radiofrequency coil used to send and collect the electromagnetic resonance signal. The coil used was small (1.5 cm diameter) and sampled or scanned only a small superficial portion of each tumor. Sample depth is approximately equal to the radius of the coil (in these experiments radius = 0.75 cm).[75,76] When a tumor increases in volume, it derives its blood supply by parasitizing blood vessels from adjacent tissue. As the tumor increases in volume the central portion of the tumor becomes increasingly distant from the supply of nutrients and oxygen carried by the vasculature. It is also this central portion of the tumor which is first to become hypoxic. It can eventually become necrotic as the lack of nutrients and oxygen causes cell death. Therefore the portion of the tumor which one would expect to become hypoxic first might not have been the portion of the tumor sampled or scanned by the radiofrequency coil used with ^{31}P NMR spectroscopy located on the surface of the tumor.[77]

An advantage of ^{31}P NMR spectroscopy is its ability to, *in-vivo*, detect and measure tumor response to therapy. Prior investigators have demonstrated that PDT interferes with normal cellular high energy phosphate metabolism by damaging cellular organelles.[24,25] In addition, PDT induces tumor hypoxia by causing microvascular collapse.[26,27,28] Both of these destructive premorbid

events produce changes in the tumor cell which should be detectable by *in-vivo* ^{31}P NMR spectroscopy. Photofrin II sensitized photodynamic therapy (PDT) of other tumor models has produced statistically significant changes in ^{31}P NMR spectra.[54,70,71]

Twenty five Dunning -AT and 28 Dunning -H tumors, of 1 cm^3 to 1.5 cm^3 in volume, were entered into the experimental protocol. Tumors underwent pre-PDT ^{31}P NMR spectroscopy the day prior to the administration of Healux H628 and photoirradiation and then again approximately one hour after PDT. Dunning -AT tumors were photoirradiated with either 100, 500, 1000, or 2000 J/cm^3, 630 nm light, over one hour while Dunning -H tumors were photoirradiated with either 100, 500, 1000, 1500, or 2000 J/cm^3, 630 nm light, also over one hour. In an attempt to measure varying grades of hypoxia in induced by PDT at different total light doses, ^{31}P NMR spectroscopy was utilized to measure relative changes in phosphorus metabolism.

After standardizing all the areas under the resonance peaks, a comparison was made between the differential values for each resonance peak at the various light doses. In the Dunning -AT tumors there were no significant statistical differences between any of the resonance peaks at any of the administered PDT light doses. From this data one might surmise that the Dunning -AT tumor is resistant to PDT at all the light energy levels used. However, we know this not to be the case. Prior work has demonstrated that both the Dunning -AT and Dunning -H tumors are responsive to interstitially applied PDT.[16,68,69] It is possible, at one hour post PDT, that the changes in

the metabolism as detected by ^{31}P NMR spectroscopy had not yet taken place. Perhaps more than one hour is required before detectable changes in the Dunning tumor's metabolism to take place. Unpublished data from the Cross Cancer Institute, Edmonton, using the sensitizer adduct technique for measuring tumor hypoxia in the Dunning -AT and -H tumor, suggest this is also not the case.[68,78] Significant hypoxia was detected within 30 minutes of PDT.[68] This suggests almost immediate changes in tumor vasculature and or tumor mitochondrial function. In addition work by Chopp, et. al., with C_3H mouse mammary carcinoma confirms the tumors altered metabolism as detected by ^{31}P NMR spectroscopy at 1 hour post PDT.[70] These researchers' data demonstrated significant alterations of resonance peaks, for both ATP and Pi, one hour post PDT.[70] These alterations also persisted at 4 and 24 hours post PDT.[70] These experimental results would suggest that if there were metabolic changes induced by PDT in the Dunning tumors, that they should have been present and measurable by ^{31}P NMR spectroscopy one hour after interstitially applied PDT.

Researchers have demonstrated significant changes in ATP and inorganic phosphorus (Pi) levels as measured by ^{31}P NMR spectroscopy after both cobalt and photodynamic therapy.[47,50,51] More recently, reports of *in-vivo* ^{31}P NMR spectroscopy detecting spectral changes in C_3H mouse mammary carcinoma after subcurative PDT, have been published.[70] Chopp, et. al., detected significant increases in Pi and decreases in cellular ATP after very low doses (25 J/cm^3) of 630 nm photoirradiation. This suggests that ^{31}P NMR spectroscopy may be a very sensitive tool in monitoring tumor response to PDT.[70] Hilf has

suggested that early increase in Pi and concomitant decreases in ATP as measured by ^{31}P NMR spectroscopy, are probably due to inhibition of mitochondrial function and maybe related to cellular hypoxia.[71] He felt that the initial and direct effect of PDT was at this cellular level and that hypoxia was secondary to a breakdown in normal cellular metabolism. He did not observe any changes in tumor vascularity, at least early in the tumors response to PDT.[71] Others have suggested, however, that the cellular hypoxia is secondary to the destruction of tumor microvasculature.[79,80]

The data presented in this thesis from experiments involving the measurement of PDT induced changes in tumor metabolism by ^{31}P NMR spectroscopy failed to demonstrate significant alterations in the phosphate spectra taken from the Dunning R3327 tumors before and after PDT. Expected changes in the ^{31}P NMR spectra after PDT failed to materialize. These results could have been secondary to a flawed experimental design. As discussed earlier in this section, other authors have reported on data which suggests that ^{31}P NMR spectroscopy is useful in detecting and measuring PDT induced changes in tumor metabolism. Perhaps the flaw in the experimental design responsible for the different results lies in the PDT portion of the protocol rather than the NMR portion of the protocol. If the outcome of the experiments reported on in this text involving ^{31}P NMR spectroscopy are secondary to improper or ineffectual implementation of the photodynamic therapy itself, then obviously there would have been no changes in the Dunning tumor's to measure with ^{31}P NMR spectroscopy. The experimental protocol governing the implementation and delivery of PDT to the Dunning tumors was previously reported to be effective

in slowing Dunning -AT and -H tumor growth, as well as producing long term cures in both tumor cell lines.[37,68,69] The technique of interstitial PDT with cylindrical irradiator fiber tips was previously reported by Arnfield, et. al., in 1984 to be an effective means of delivering light energy for PDT.[65] Since the initial reports using the experimental methodology similar to that described earlier in this text, the methodology has been carefully examined and refined and has already been used to successfully treat Dunning tumors.[37,68,69] Therefore, it seems extremely unlikely that the unexpected, unchanged post-PDT ^{31}P NMR spectra taken from Dunning tumors were due to an error in the implementation and delivery of PDT.

There was one single variable which had been altered from the previously reported PDT protocols from this lab.[37,65,68,69] The unavailability of Photofrin II necessitated a search for an alternative photosensitizer. Healux H628 was selected as an alternate photosensitizer based on reports of its chemical composition and its experimental efficacy.[64] Dihematoporphyrin ether of ester (DHE) or a polymer of that chemical, is thought to be the active component of both Photofrin II and healux H628.[8,10,11,64] Lai, et. al., used high pressure liquid chromatography (HPLC) to compare the relative amounts of DHE in both Healux H628 and Photofrin II. Healux H628 was apparently 92% DHE as apposed to Photofrin II which is 22% DHE.[64] In addition Healux H628 was more stable requiring only dark, room temperature storage versus the -20°C dark storage required by Photofrin II.[64] Lai, et. al., also published tumor response data, both in-vivo, and in-vitro, comparing Healux H628 and Photofrin II. Tumor response was as good, if not better with Healux H628 photosensitized

PDT than with Photofrin II photosensitized PDT.[64] Because of this experimental evidence there was no prior reason to suspect Healux H628's efficacy. However, there was no experimental data using this compound with interstitial photoirradiation in either of the Dunning tumor lines and unfortunately a prospective comparison with Photofrin II was impossible because of Photofrin II's unavailability.

In April 1989 Photofrin II became available again for experimental protocols from Quadra Logic Inc., Vancouver, B.C.. This allowed controlled comparative studies between Healux H628 and Photofrin II to be carried out. Two separate comparative experiments were performed and were already described in greater detail earlier in this text. The first experiment involved a comparison of ^{31}P NMR spectroscopy before and after PDT with either Photofrin II or Healux H628. The experimental protocol was exactly the same as that used for the previous NMR studies. Only the Dunning - AT tumor was used in these comparative studies. Each animal with it's respective Dunning - AT tumor acted on its own control by undergoing ^{31}P NMR spectroscopy 24 hours prior to photosensitization and photoirradiation. Each group received the same mg/kg dose of either Healux H628 or Photofrin II as the photosensitizer and then 4 hours later received 2000 J/cm^3 of 630 nm light photoirradiation. ^{31}P NMR spectroscopy was repeated approximately 1 hour post PDT in both groups. Again the average area under each resonance peak was standardized, averaged for the entire experimental group and then compared pre and post PDT.

As noted previously there were no significant differences in the ^{31}P NMR spectra comparing pre and post-PDT when Healux H628 was used as the photosensitizer. However, there were significant differences in the ^{31}P NMR spectra between pre and post PDT when Photofrin II was used as the photosensitizer. These results with Photofrin II were more in keeping with what was originally expected when the ^{31}P NMR protocol was developed. Significant decreases in the three ATP moieties, alpha, gamma and beta, occurred in all of the ^{31}P NMR spectra taken from Dunning -AT tumors treated with Photofrin II sensitized PDT. These results were similar to those reproted on by Hilf and Chopp.[47,54.70,71] They suggested that decreased levels of measurable ATP were the result of PDT induced mitochondrial damage.[47,54.70,71] Other authors have also documented decreases in the ATP resonance peaks as a response to radiation induced ischemia.[53] Okunieff, et. al., showed that decreased oxygen saturation in murine tumors also resulted in the lowering of cellular ATP as measured by ^{31}P NMR spectroscopy.[52] In addition all of the tumors treated by PDT using Photofrin II had a significant increase in measured inorganic phosphorous (Pi).[53,70,71] The increase in Pi is thought to be due to ischemia and resultant tissue necrosis and occurs concurrently with the fall in measurable ATP.[47,71]

The data from experiments reported on in this paper suggested that there is a significant statistical difference between Healux H628 and Photofrin II in terms of their photosensitizing ability and PDT's resulting ability to induce tumor ischemia and hypoxia as measured by ^{31}P NMR spectroscopy. At this point the efficacy of Healux H628 as a photosensitizer was questioned. This

prompted a second comparative experiment measuring the delay in tumor regrowth after PDT using either Healux H628 or Photofrin II. The Dunning AT tumor is known to be more resistant to all forms of therapy.[37,63,68,69] Photodynamic therapy (1000 J/cm^3) was combined with misonidazole in order to potentiate the PDT response in both experimental groups.[37] The two treatment groups were compared to each other and to untreated controls. The experimental end point for the study was growth to >10x initial measured volume in the control group and regrowth to >10x treatment volume in the Healux H628 and Photofrin II treated groups. In both treatment groups tumor regrowth was significantly delayed when compared to the untreated control group (analysis of variance, Bonferroni t-test, $P<0.01$) (figure #2). The delay in tumor regrowth (mean standard deviation time to regrowth to >10x treatment volume) after combination MISO and PDT sensitized by Healux H628 (20.56 6.6 days) was statistically significantly less than the delay in regrowth seen after combination MISO and PDT sensitized by Photofrin II (29.75 8.8 days) (analysis of variance, Bonferroni t-test, $P<0.01$).

These two experiments, one using ^{31}P NMR spectroscopy to measure tumor response after PDT sensitized with either Healux H628 or Photofrin II and one measuring tumor volume regrowth after PDT sensitized with either Healux H628 or Photofrin II, compare the efficacy of Healux H628 and Photofrin II as photosensitizers and clearly show that Healux H628 is a significantly less effective photosensitizer (at least in the experimental models used in this paper). Therefore, the results obtained in experiments presented in

this document, which deviated from what was expected, were most likely due to the use of a significantly inferior photosensitizing agent, Healux H628.

Results from the experiments comparing the efficacy of Healux H628 and Photofrin II as photosensitizer agent has a significant bearing on the conclusions that can be made from the ^{31}P NMR spectroscopy data, collected after varied doses of PDT sensitized with Healux H628. As already discussed the study comparing ^{31}P NMR spectroscopy after PDT (2000 J/cm^3) with either Healux H628 or with Photofrin II documented spectral changes after PDT with Photofrin II which did not occur after PDT with Healux H628. The absence of spectral change is most certainly secondary to Healux H628's inability to adequately photosensitize the tumor prior to photoirradiation. Had the ^{31}P NMR spectroscopy pre- and post-PDT protocol utilized Photofrin II it is almost certain that the results would have been significantly different. Tumor response to graded increases in light energy during PDT would have been represented on ^{31}P NMR spectroscopy by gradual decreases in ATP resonance peaks and concurrent increases in Pi resonance peaks. It is very possible that ^{31}P NMR spectroscopy is quite sensitive in detecting PDT induced tumor hypoxia and ischemia and that further experiments may show it to be quite useful in measuring and even possibly predicting tumor response to therapy.

Investigators undertaking clinical trials utilizing PDT have in the past waited 24 to 72 hours between the administration of the photosensitizer agent and the application of photoirradiation.[9,11] Since HpD seems to be selectively retained in neoplastic tissue this delay maximized the difference in concentration

of the photosensitizer between histologically normal and malignant tissue.[34,55] A delay of 24-72 hours after the administration of photosensitizer might improve the therapeutic ratio of PDT, in terms of normal tissue sparing, but it might also have a detrimental effect on PDT's tumoricidal effect. Maximal concentration of photosensitizers, such as hematoporphyrin derivative (HpD), dihematoporphyrin ether (DHE, Photofrin II) or polyhematoporphyrin ester (Healux H628), occurs at 4-6 hours after administration, as determined by fluorescent analysis.[32,37] Thus, a study already described earlier in greater detail was undertaken comparing delay in tumor regrowth after PDT 4 hours post-photosensitization vs 24 hours post-photosensitization with Photofrin II. The control group of 18 F_1 hybrid male rats, received no sensitizer and no PDT. The resultant control growth curve demonstrated rapid tumor growth, as expected from this anaplastic tumor. The mean (± standard deviation) growth time to >10x their initial measured volume was 16.06 ± 3.03 days. Tumor response in both therapeutic groups showed a significant delay compared to controls (analysis of variance, Bonferroni t-test, $P<0.01$). There is no doubt that interstitially applied PDT can produce a delay in Dunning -AT tumor regrowth and even produce cures in some instances.[37] With interstitial PDT normal tissue sparing occurs because of precise positioning of the photoirraditor tips.[65] There is a significant drop off in light intensity which occurs at a distance greater than 5-9 mm from the photoirradiator tip.[57] The ability to spare normal tissue by controlling the photodosimetry should compensate for the normal tissue sparing effect of delivering the photoirradiation 24-72 hours after photosensitization. It was hoped that photoirradiation 4 hours after the administration of the photosensitizer, when tissue concentration of the

photosensitizer peaked, might significantly delay tumor regrowth when compared to the delay of tumor regrowth in the 24 hour group. Indeed, in the 11 F_1 hybrid rats which were photoirradiated 4 hours after the administration of Photofrin II, there were 3 long term cures (where there was no palpable tumor 30 days after treatment). There was only one long term cure in the 24 hour group but the difference between groups was not statistically significant. In addition, a significantly prolonged delay in tumor regrowth was not demonstrated in the 4 hour group when compared to the 24 hour group. Subjectively, there did not appear to be any greater degree of normal tissue sparing in the 24 hour group when compared to the 4 hour group. Since in this setting of interstitial PDT normal tissue sparing is dictated by photodosimetry, there seems to be no benefit in waiting 24 hours after photosensitization before proceeding with PDT. However, no advantage in terms of tumor response could be delineated by photoirradiation 4 hours post-photosensitization.

Protocols reported on by Hirsch, et. al., and Gonzales, et. al., of Dunning R3327 -AT tumors treated by PDT (Photofrin II, 15 mg/kg body wt., 2160 J/cm^3) can be used for comparison.[37,68] There was no statistically significant difference (analysis of variance, Bonferroni t-test, $P=0.33$) between control growth rates reported on in this paper and the control growth rates of tumors reported on by Hirsch.[68] However, control growth rates as reported by Gonzales, et. al., were statistically significantly faster (analysis of variance, Bonferroni t-test, $P<0.001$) than either of the control tumor growth rates as reported here or by Hirsch.[37,68] In the experimental protocols used by Hirsch, et.al., and Gonzales, et. al., the mean (± standard deviation) tumor volume at

the onset of their respective protocols were similar.[37,68] However, this was statistically significantly (analysis of variance, Bonferroni t-test, $P<0.05$) smaller than the mean (± standard deviation) tumor volume used in the protocol from this thesis. The faster growth rate in the series described by Gonzales, et. al., may have been due to differences in the degree of anaplasia of his Dunning -AT tumors. The Dunning -AT is known to "drift" and can become more anaplastic with successive tumor transfers between generations of male F_1 hybrid rats.[59,60,61]

The treatment group reported on by Hirsch had the greatest response to therapy with a mean (± standard deviation) time to regrowth of 31.5 ± 8.54 days.[68] This delay in tumor regrowth was statistically longer (analysis of variance, Bonferroni t-test, $P<0.001$) than the delay seen in either treatment group as reported upon in this paper or by Gonzales, et. al..[37] The delay in tumor regrowth as reported in this paper was significantly longer (analysis of variance, Bonferroni t-test, $P<0.05$) than the delay in tumor regrowth reported on by Gonzales, et. al., (mean ± standard deviation of 21.75 ± 4.33 days and 17.2 ± 5.35 days, respectively). The significantly faster regrowth in the series reported on by Gonzales, et. al., maybe related to inherent biological factors of the tumor line or may be due to refinements in the application of PDT that have evolved since publication of their results. The capability of the Dunning -AT tumor to de-differentiate and become more anaplastic with faster doubling times could have played a significant role in the tumor's variable response to PDT. The more anaplastic variant would obviously be more resistant to any form of treatment. There have also been substantial improvements made in the

application of PDT since the publication of the paper by Gonzales, et. al.. Specifically improvements in positioning of the fiber optic tips and improved photodosimetry might account for the better tumor response in the more recent protocols by Hirsch, et. al., and as reported on in this paper.

The superior tumor response documented by Hirsch, et. al., when compared to the response documented in this series, might be most plausibly explained by differences in the mean tumor volume of the two respective treatment groups. The significantly smaller tumors treated by Hirsch, et. al., would obviously respond better to therapy than the larger tumors used in this series. For example a larger measure of light energy (J/cm^3) would be absorbed by the smaller tumors (used by Hirsch, et. al.) when compared to the larger tumors (used in this paper.) Otherwise, the experimental protocols were almost identical in every respect.

The last experimental protocol to be discussed was designed to compare the Dunning R3327 -AT tumor's response to treatment with newer hypoxic cytotoxic agents (RSU-1069 and SR-4233), or misonidazole, in combination with PDT. Each of these drugs has been shown to be directly cytotoxic to hypoxic cells.[43,44,45] Using *in-vitro* studies, SR-4233 and RSU-1069 were shown to be approximately 100x and 300x respectively, more cytotoxic than misonidazole.[45] Prior experimentation in the Dunning tumor model documented that radiotherapy in combination with misonidazole was able to produce significantly longer delays in tumor radiation alone.[63] Further work by Gonzales, et. al., and

Hirsch, et. al., suggested that misonidazole also potentiated the Dunning -AT tumor's response to PDT.[37,68]

Untreated controls (n = 10) were compared to groups treated with PDT in combination with misonidazole (n = 9), RSU-1069 (n = 8) or SR-4233 (n = 5). All three treatment groups produced significant delays in tumor regrowth when compared to untreated controls (analysis of variance, Bonferroni t-test, $P < 0.01$). SR-4233, in combination with PDT, produced a longer delay in tumor regrowth than did combination RSU-1069 and PDT, which in turn produced a longer delay in tumor regrowth than did combination misonidazole and PDT. None of these differences between treatment groups were statistically significant. However, 3 of the eight animals treated with combination PDT and SR-4233 died, presumably because of the systemic toxicity of SR-4233 (at the specific dose of SR-4233 used). None of the animals in either of the other treatment groups died suggesting that SR-4233 was more systemically toxic than either RSU-1069 or misonidazole (at the respective doses used in this experimental protocol).

Gonzales et. al. demonstrated a significant delay in tumor regrowth when the radiant energy used in PDT was increased from 1224 J/cm^3 to 2376 J/cm^3.[37] His first group was a control group which received no therapy and no drugs. The tumors in this animal group were followed from an initial volume of 500-1000 mm^3, until they grew to 10x that initial volume. The second group were given Photofrin II, 15 mg/kg body wt, 4 hr prior to PDT at a total dose of 1224 J/cm^3. The tumors in this animal group were followed with tumor volume

measurements until they regrew to 10x their treatment volume. The tumor growth delay in these experimental groups were then compared to groups which received combination PDT and hypoxic cytotoxic therapy with MISO. This data demonstrated that PDT alone at a low dose of 1224 J/cm^3 was unable to produce as long a delay in regrowth as PDT in combination with hypoxic cytotoxic therapy. Hirsch et. al. confirmed that increasing radiant light energy further delayed tumor regrowth.[68] At a dose of radiant energy greater than 1000 J/cm^3 the degree of tumor hypoxia induced by PDT is significantly greater.[37,68] The larger fraction of hypoxic cells present after PDT with greater doses of radiant energy may allow for a greater direct cellular cytotoxic effect by the adjuvant hypoxic agents. A more powerful light dose may have resulted in a larger difference between the three treatment groups, in terms of tumor response.

In addition it has been shown earlier in this text that Healux H628 is a less effective or less efficient photosensitizer than Photofrin II, at least in this tumor model. In this particular experiment PDT sensitized with Healux H628 may not have induced enough tumor hypoxia to allow for maximal effect by each of the respective hypoxic cytotoxic agents. The differences in tumor regrowth may have been much more significant if Photofrin II had been used as a photosensitizer.

CONCLUSION:

As proven by the results of experiments described in this thesis, Healux H628 is a less efficacious or is an inferior photosensitizing agent when compared to Photofrin II. This fact has a significant impact on the conclusions that can be derived from the results presented in this paper.

^{31}P NMR spectroscopy is able to measure significant differences in phosphorous metabolism between the poorly differentiated Dunning -AT tumor and the well differentiated Dunning -H tumor. However it was unable to detect differences in phosphorous metabolism between small and large tumors of the same tumor line. In addition there were no detectable changes in phosphorous metabolism in either tumor line after PDT at graded increasing light doses of photoirradiation. This was however not due to the inability of ^{31}P NMR spectroscopy to detect changes in phosphorous metabolism after PDT but was likely due to the ineffectiveness of Healux H628 and its inability to photosensitize the tumors adequately prior to photoirradiation.

The technique of interstitial photoirradiation utilizes photodosimetry to provide normal tissue sparring and deliver an acceptable therapeutic light dose. Altering the length of time between the administration photosensitizer (Photofrin II) and photoirradiation failed to change the tumor response. There also seemed to be little correlation between the temporal relationship of photosensitizer administration to photoirradiation with the interstitial technique and the degree of normal tissue sparing.

Combination PDT with hypoxic cytotoxic agents such as MISO, RSU-1069 and SR-4233 produced significant delays in Dunning -AT tumor regrowth when compared to the growth of a control group of tumors. However, at a light dose of 1000 J/cm^3 and using Healux H628 as the photosensitizer, no significant difference in tumor regrowth was noted between the groups treated with the different hypoxic cytotoxic agents. The combination of the lower light dose and the less effective photosensitizer agent may not have induced enough tumor cell hypoxia to allow the respective hypoxic cytotoxic agents to work to their full capacity. At the doses used, SR-4233 was more toxic to the F_1 hybrid male rats (from Fischer (female) x Copenhagen (male) matings) than either RSU-1069 or MISO.

Photodynamic therapy sensitized with Photofrin II was able to produce significant changes in post-PDT ^{31}P NMR spectra of Dunning -AT tumors compared to the tumor's respective pre-PDT ^{31}P NMR spectra. PDT sensitized with Photofrin II was able to produce significantly greater delays in Dunning -AT tumor regrowth when compared to untreated controls and when compared to tumors treated with PDT sensitized by Healux H628. There is little question that, at least in this tumor and experimental model, Photofrin II is a superior photosensitizing agent compared to Healux H628.

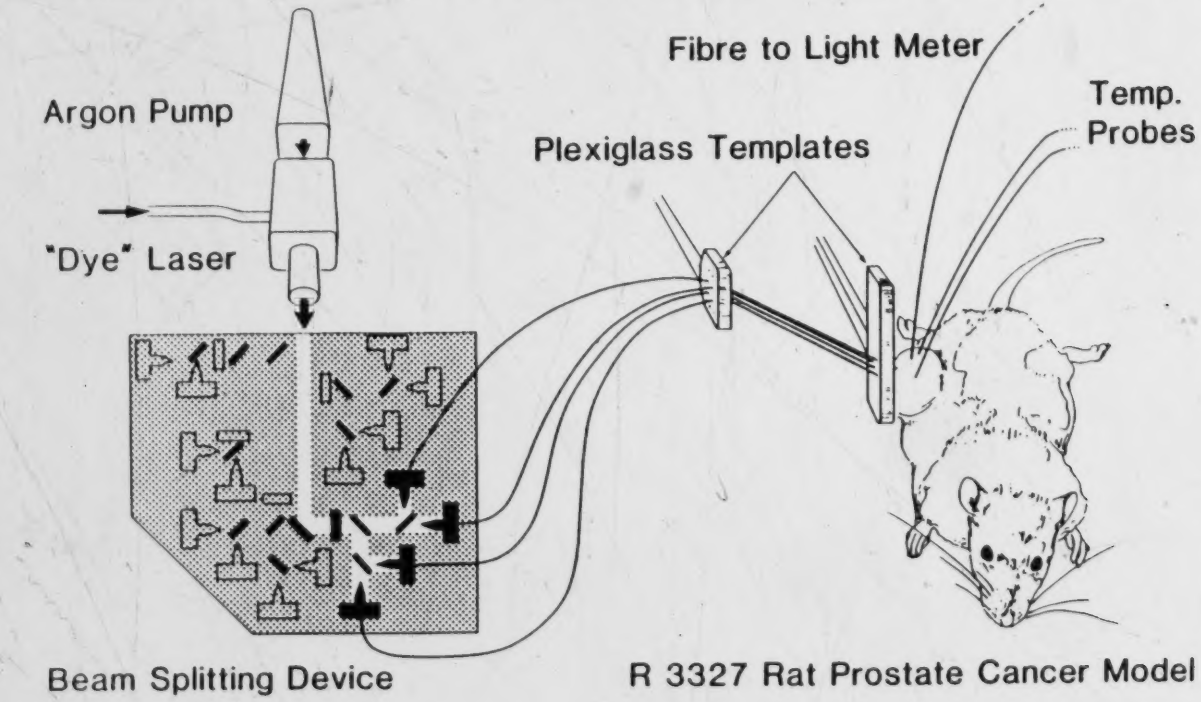
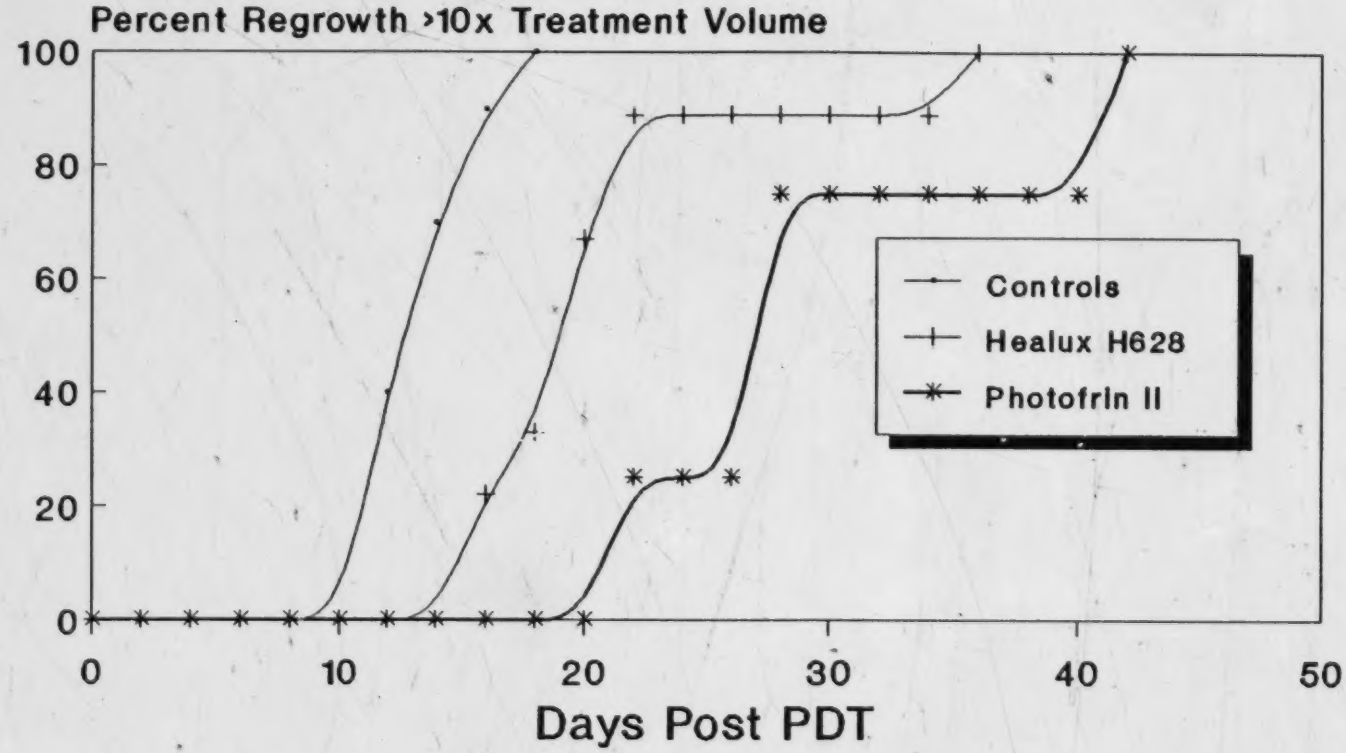


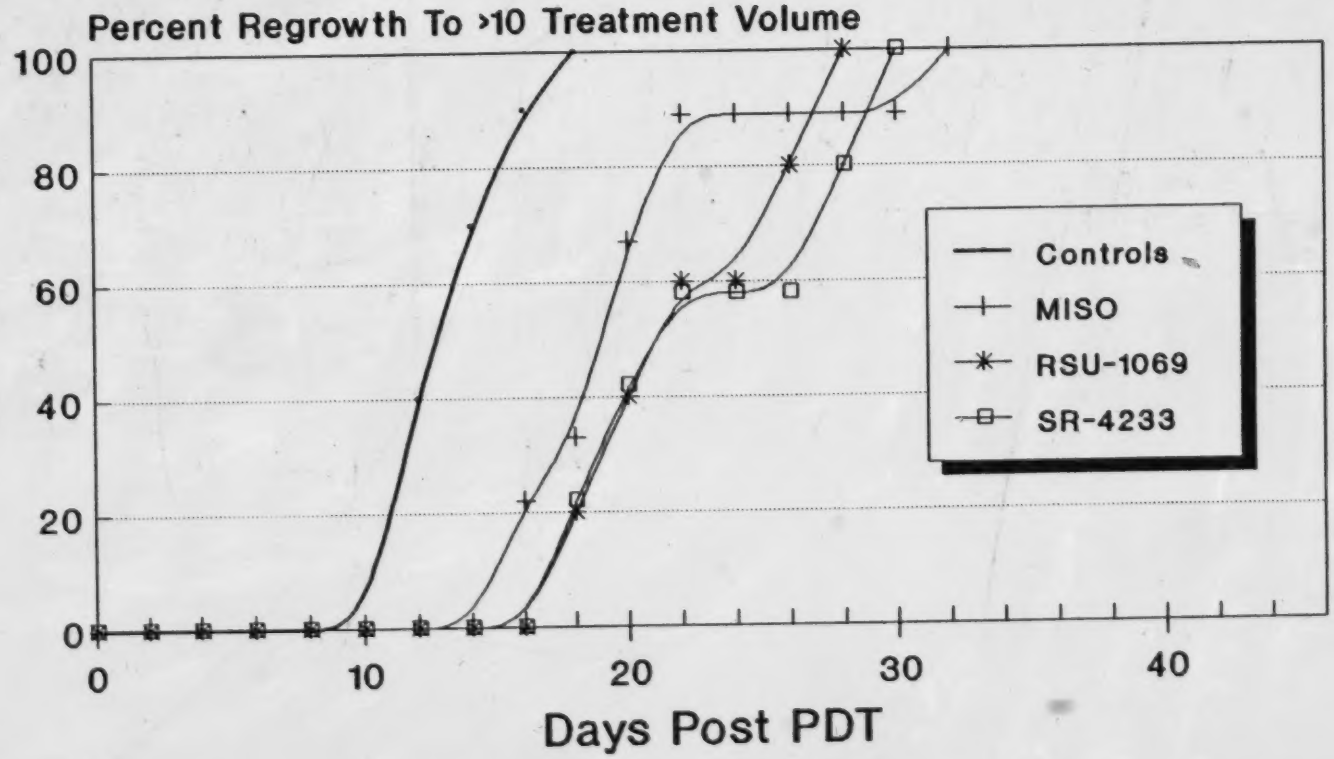
FIGURE #1: DIAGRAM OF THE EXPERIMENTAL DESIGN USED FOR INTERSTITIAL PHOTODYNAMIC THERAPY IN THE DUNNING R 3327 RAT PROSTATE CANCER MODEL.

Figure # 2:



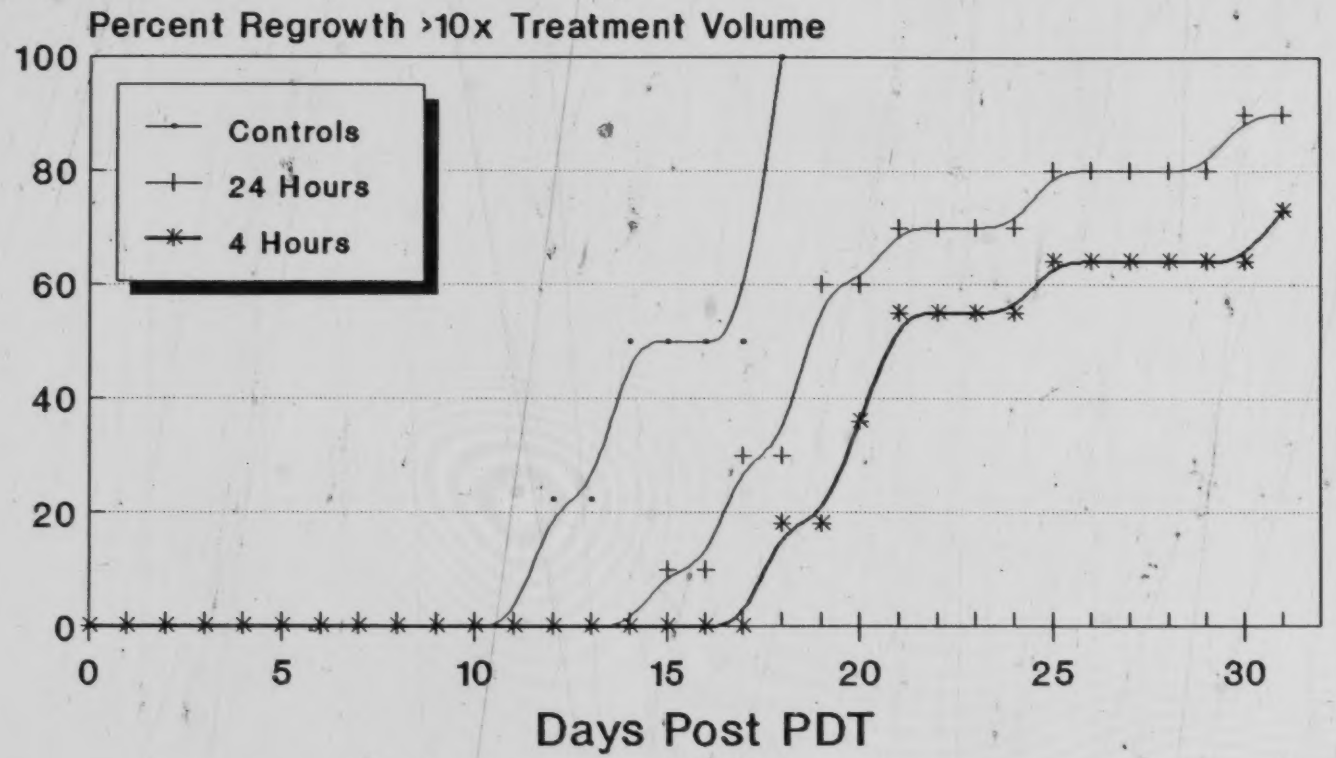
Delay in tumor regrowth differences after PDT photosensitized with either Healux H628 or Photofrin II.

Figure #3:



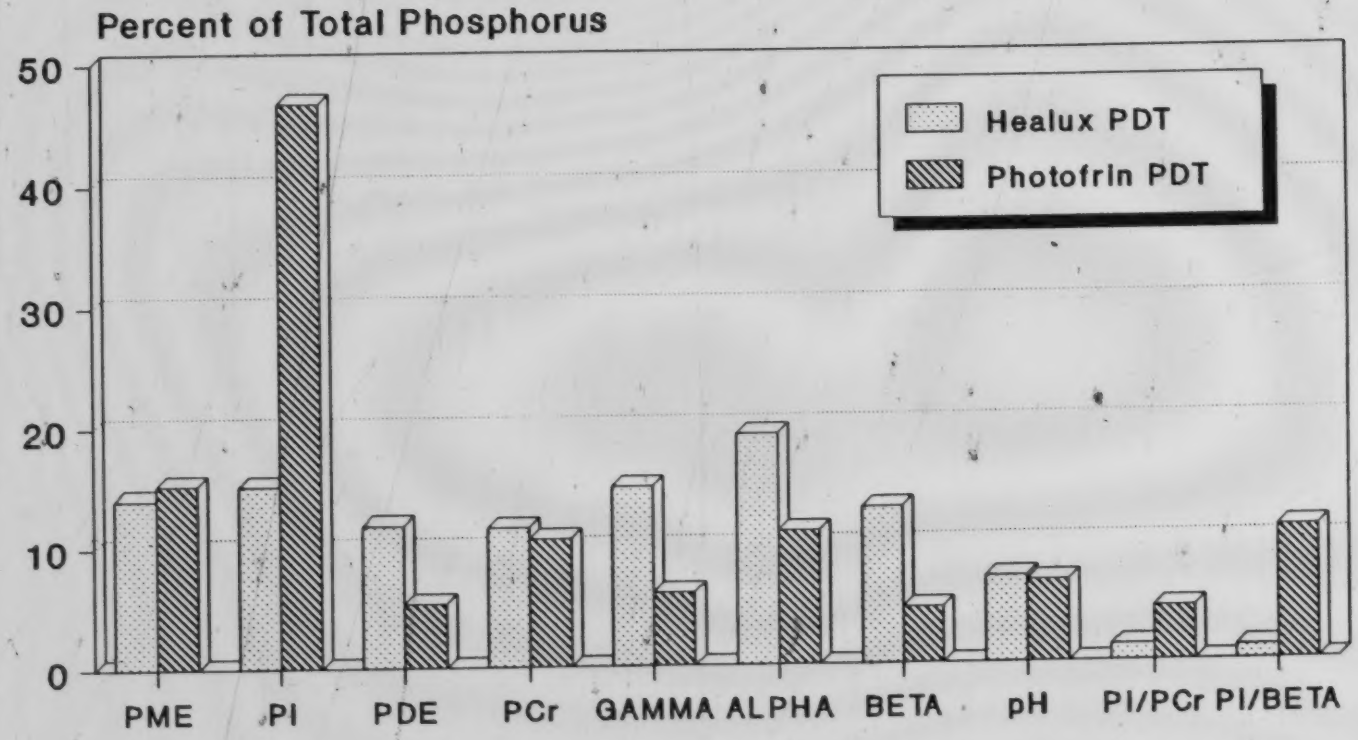
Delay in tumor regrowth, Dunning -AT tumor after combination MISO, RSU-1069, SR-4233 and PDT (1000J/cc, Healux H628)

Figure #4:



Delay in tumor regrowth, Dunning -AT tumor, post-PDT, photosensitizer given 4 hr or 24 hr prior to PDT (2000 J/cc)

Figure #5:



Relative comparison of phosphorus signal after PDT (2000J/cc) photosensitized with either Healux H628 or Photofrin II.

TABLE 1. CONTROL 31P NMR SPECTRA, DUNNING -H TUMOR.

RAT #	VOL (cu. mm.)	PME	Pi	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
2871	3992	16.5	7.9	14.5	14.4	13.4	19.7	13.5
2872	3804	16.2	9.3	13.1	19.6	13.2	17.1	11.5
2873	2792	15.8	8.6	11.1	18.0	12.0	19.6	15.0
2881	1149	14.7	4.6	14.2	17.6	14.9	20.6	13.5
2876	2831	11.9	14.3	12.8	20.1	11.2	20.0	9.7
2894	3125	14.5	7.4	8.5	23.9	12.0	18.3	15.4
2878	3422	14.8	9.0	12.0	18.7	9.1	18.4	17.9
2888	3930	22.3	4.7	9.5	17.9	9.7	22.9	12.9
2898	1222	12.6	10.0	14.7	16.8	13.8	19.8	12.2
2922	1080	15.6	6.6	14.3	12.6	12.2	21.8	16.9
2925	1393	13.7	7.5	10.1	16.8	14.2	21.0	16.7
2924	1081	14.1	10.6	14.2	16.1	14.1	18.4	12.5
2870	2707	17.8	10.5	11.3	18.9	12.0	17.3	12.2
2980	1059	13.1	8.0	14.0	15.6	14.8	18.0	16.5
2971	1266	13.9	9.7	10.8	22.9	11.7	18.7	12.3
2972	955	13.7	10.0	14.8	12.5	13.2	20.8	15.0
2974	953	16.5	6.6	15.7	14.9	12.9	19.5	13.9
2983	1063	18.0	7.2	7.9	25.0	9.5	18.4	14.0
3004	1632	14.9	13.0	9.9	16.5	13.9	19.5	12.3
3008	1238	12.1	7.5	15.2	17.2	12.7	20.5	14.8
3009	2175	13.4	12.6	15.7	12.4	15.6	17.6	12.7
3011	1249	20.0	7.4	12.2	11.5	13.7	19.7	15.5
2979	1837	15.0	13.5	13.7	12.8	12.7	18.8	13.5
2975	1320	15.7	6.6	12.6	18.9	12.0	20.5	13.7
2977	1723	13.4	7.3	14.2	15.0	14.3	19.8	16.0
3001	1521	16.9	10.1	13.1	8.9	14.8	21.4	14.9
3012	1664	14.5	9.1	11.7	13.7	15.6	19.8	15.6
3005	1086	15.7	7.6	13.0	14.8	13.6	21.2	14.2
MEAN:	1902.5	15.3	8.8	12.7	16.6	13.0	19.6	14.1
SD:	994.8	2.3	2.4	2.1	3.7	1.7	1.4	1.9

Shown are relative amounts of phosphate monoester (PME),inorganic phosphate (Pi), phosphate diester(PDE), phosphocreatine (PCr), Gamma, Alpha and Beta Adenosine triphosphate (ATP).

TABLE 2. CONTROL 31P NMR SPECTRA, DUNNING -H TUMOR.

RAT #	VOL (cu. mm.)	PME	Pi	PDE	PCr	GAMMA ATP:	ALPHA ATP:	BETA ATP:
2974	953	16.5	6.6	15.7	14.9	12.9	19.5	13.9
2972	955	13.7	10.0	14.8	12.5	13.2	20.8	15.0
2980	1059	13.1	8.0	14.0	15.6	14.8	18.0	16.5
2983	1063	18.0	7.2	7.9	25.0	9.5	18.4	14.0
2922	1080	15.6	6.6	14.3	12.6	12.2	21.8	16.9
2924	1081	14.1	10.6	14.2	16.1	14.1	18.4	12.5
3005	1086	15.7	7.6	13.0	14.8	13.6	21.2	14.2
2881	1149	14.7	4.6	14.2	17.6	14.9	20.6	13.5
2898	1222	12.6	10.0	14.7	16.8	13.8	19.8	12.2
3008	1238	12.1	7.5	15.2	17.2	12.7	20.5	14.8
3011	1249	20.0	7.4	12.2	11.5	13.7	19.7	15.5
2971	1266	13.9	9.7	10.8	22.9	11.7	18.7	12.3
2975	1320	15.7	6.6	12.6	18.9	12.0	20.5	13.7
2925	1393	13.7	7.5	10.1	16.8	14.2	21.0	16.7
3001	1521	16.9	10.1	13.1	8.9	14.8	21.4	14.9
3004	1632	14.9	13.0	9.9	16.5	13.9	19.5	12.3
3012	1664	14.5	9.1	11.7	13.7	15.6	19.8	15.6
2977	1723	13.4	7.3	14.2	15.0	14.3	19.8	16.0
2979	1837	15.0	13.5	13.7	12.8	12.7	18.8	13.5
3009	2175	13.4	12.6	15.7	12.4	15.6	17.6	12.7
2870	2707	17.8	10.5	11.3	18.9	12.0	17.3	12.2
2873	2792	15.8	8.6	11.1	18.0	12.0	19.6	15.0
2876	2831	11.9	14.3	12.8	20.1	11.2	20.0	9.7
2894	3125	14.5	7.4	8.5	23.9	12.0	18.3	15.4
2878	3422	14.8	9.0	12.0	18.7	9.1	18.4	17.9
2888	3930	22.3	4.7	9.5	17.9	9.7	22.9	12.9
MEAN:	1749.0	15.2	8.8	12.6	16.5	12.9	19.7	14.2
SD:	851.5	2.4	2.5	2.2	3.8	1.8	1.4	1.9

Tumors are listed in order of increasing volume. Shown are relative amounts of phosphate monoester (PME),inorganic phosphate (Pi), phosphate diester(PDE), phosphocreatine (PCr), Gamma, Alpha and Beta Adenosine triphosphate (ATP).

TABLE 3. COMPARISON OF CONTROL P31 NMR SPECTRA, SEVEN SMALLEST AND FIVE LARGEST DUNNING -H TUMORS.

31P NMR SPECTRA, SEVEN SMALLEST DUNNING -H TUMORS.

RAT #	VOL: (cu. mm.)	PME	Pi	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
2974	953	16.5	6.6	15.7	14.9	12.9	19.5	13.9
2972	955	13.7	10.0	14.8	12.5	13.2	20.8	15.0
2980	1059	13.1	8.0	14.0	15.6	14.8	18.0	16.5
2983	1063	18.0	7.2	7.9	25.0	9.5	18.4	14.0
2922	1080	15.6	6.6	14.3	12.6	12.2	21.8	16.9
2924	1081	14.1	10.6	14.2	16.1	14.1	18.4	12.5
3005	1086	15.7	7.6	13.0	14.8	13.6	21.2	14.2
MEAN:	1039.6	15.2	8.1	13.4	15.9	12.9	19.7	14.7
SD:	59.3	1.7	1.6	2.6	4.2	1.7	1.5	1.5

31P NMR SPECTRA, FIVE LARGEST DUNNING -H TUMORS.

RAT #	VOL: (cu. mm.)	PME	Pi	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
2894	3125	14.5	7.4	8.5	23.9	12.0	18.3	15.4
2878	3422	14.8	9.0	12.0	18.7	9.1	18.4	17.9
2872	3804	16.2	9.3	13.1	19.6	13.2	17.1	11.5
2888	3930	22.3	4.7	9.5	17.9	9.7	22.9	12.9
2871	3992	16.5	7.9	14.5	14.4	13.4	19.7	13.5
MEAN:	3654.6	16.9	7.7	11.5	18.9	11.5	19.3	14.2
SD:	369.7	3.2	1.8	2.5	3.4	2.0	2.2	2.5

Shown are relative amounts of phosphate monoester (PME),inorganic phosphate (Pi), phosphate diester(PDE), phosphocreatine (PCr), Gamma, Alpha and Beta Adenosine triphosphate (ATP). There is a statistically significant difference between the mean volumes of the small and large groups (analysis of variance, Bonferroni t-test, $P<0.001$).

TABLE 4. TREATMENT 31P NMR SPECTRA, DUNNING -H TUMOR.

RAT #	J/cc	VOL (cu. mm.)	PME	Pi	PDE	PCr	GAMMA ATP:	ALPHA ATP:	BETA ATP:
2871	100	3992	14.4	13.2	16.0	10.1	15.5	19.1	11.6
2872	100	3804	17.8	21.9	3.8	19.4	8.9	17.5	10.7
2873	100	2792	14.9	11.7	8.7	20.3	11.5	19.2	13.6
2881	100	1149	17.7	26.8	5.8	16.2	10.0	16.0	7.6
2876	100	2831	16.4	18.9	8.1	16.0	8.9	18.9	12.9
MEAN		2913.6	16.2	18.5	8.5	16.4	11.0	18.1	11.3
SD:		1128.2	1.6	6.2	4.6	4.0	2.8	1.4	2.3
2894	500	3125	11.1	14.9	13.0	14.5	13.4	19.2	13.9
2878	500	3422	15.8	17.4	11.5	14.3	13.3	17.5	10.4
2888	500	3930	18.5	12.2	8.0	14.5	12.2	19.5	15.1
2898	500	1222	11.4	17.2	5.0	24.3	11.8	18.6	11.7
2922	500	1080	17.9	10.5	14.6	11.2	13.4	18.4	14.0
2925	500	1393	16.4	15.1	10.3	14.6	14.0	18.8	10.8
2924	500	1081	16.4	11.8	11.0	17.4	13.2	19.0	11.3
2870	500	2707	20.9	19.6	9.1	13.8	11.3	13.1	12.3
MEAN:		2245.0	16.1	14.8	10.3	15.6	12.8	18.0	12.4
SD:		1177.0	3.4	3.2	3.0	3.9	0.9	2.1	1.7
2980	1000	1059	15.0	22.7	6.8	15.2	12.3	17.7	10.3
2971	1000	1266	16.4	19.6	9.4	14.4	9.1	16.4	14.7
2972	1000	955	15.0	14.4	8.8	19.5	13.9	17.3	11.1
2974	1000	953	14.0	12.9	6.0	23.3	10.7	19.1	14.0
2983	1000	1063	15.5	12.2	12.3	17.6	12.0	17.2	13.3
MEAN:		1059.2	15.2	16.4	8.7	18.0	11.6	17.5	12.7
SD:		127.4	0.9	4.6	2.5	3.6	1.8	1.0	1.9
3004	1500	1632	19.0	23.6	4.5	5.1	20.7	17.1	10.1
3008	1500	1238	16.8	9.5	12.4	23.5	7.6	16.4	13.8
3009	1500	2175	22.0	9.1	14.3	10.3	12.5	18.2	13.6
3011	1500	1249	25.5	12.1	21.5	6.0	8.5	13.1	13.3
MEAN:		1573.5	20.8	13.6	13.2	11.2	12.3	16.2	12.7
SD:		440.9	3.8	6.8	7.0	8.5	6.0	2.2	1.7
2979	2000	1837	16.8	17.5	11.2	11.6	12.4	18.9	11.8
2975	2000	1320	17.7	9.4	19.8	11.6	12.7	17.4	11.4
2977	2000	1723	18.6	15.9	6.7	16.1	12.3	19.1	11.4
3001	2000	1521	22.1	10.4	9.1	12.8	13.9	18.8	12.9
3012	2000	1664	19.5	31.0	10.3	9.0	7.8	14.6	7.7
3005	2000	1086	22.3	23.3	10.9	8.2	11.2	15.2	8.9
MEAN:		1525.2	19.5	17.9	11.3	11.6	11.7	17.3	10.7
SD:		279.5	2.3	8.2	4.5	2.8	2.1	2.0	2.0

Presenting the light dose at which the tumors were treated (J/cc), and relative amounts of phosphate monoester (PME), inorganic phosphate (Pi), phosphate diester (PDE), phosphocreatine (PCr), Gamma, Alpha and Beta Adenosine triphosphate (ATP). Tumors are grouped according to the total light dose used during PDT.

TABLE 5. CONTROL 31P NMR SPECTRA, DUNNING -AT TUMORS.

RAT #	VOL: (cu. mm.)	PME	PI	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
2780	1580	13.3	14.6	14.1	14.4	11.3	18.6	13.7
2830	1054	5.5	10.7	1.7	32.0	14.4	20.3	15.4
2848	4558	16.3	14.4	11.2	16.3	11.2	17.8	12.7
2856	4932	14.6	25.0	7.7	12.7	12.4	18.0	9.7
2910	1177	15.1	17.3			12.4	20.0	12.7
2913	1489	15.7	14.1	7.9	13.4	12.9	20.9	15.0
2917	1605	11.0	14.7	8.9	17.4	14.3	20.2	13.6
2907	1164	14.0	12.9	12.4	13.2	14.2	19.9	13.4
2912	1942	15.6	12.8	15.0	9.1	15.4	19.6	12.3
2908	6975	13.9	15.0	11.8	11.6	15.2	17.2	15.3
2914	7950	15.1	14.4	14.8	6.8	14.7	19.1	15.2
2919	5117	19.8	17.7	8.9	6.3	13.6	20.7	13.0
2926	1682	15.3	14.4	10.5	11.8	13.9	19.7	14.5
2930	1277	15.2	14.4	13.2	10.7	12.8	20.6	13.0
2931	1206	15.2	17.2	12.6	13.4	13.3	17.3	10.9
2937	1363	11.5	12.9	13.3	12.5	14.8	21.4	13.6
2939	1137	15.3	18.7	6.8	18.9	10.5	18.0	12.0
2951	1369	16.1	12.8	14.6	9.1	14.0	18.9	14.5
2945	1812	14.2	20.3	13.4	12.8	10.5	17.1	11.8
2964	1636	12.1	17.5	7.4	15.2	14.2	18.8	14.8
3000	1419	12.6	10.6	12.2	12.7	17.2	19.6	15.1
2997	1036	14.2	13.9	7.3	13.2	15.8	21.5	14.1
2999	1178	16.5	15.5	12.0	9.9	14.6	17.2	14.3
2994	1535	14.2	15.0	10.0	10.3	13.7	22.1	14.7
2992	1434	14.5	15.3	10.1	12.4	13.3	19.6	14.8
MEAN:	2305.1	14.3	15.3	10.7	13.2	13.6	19.4	13.6
SD:	1947.2	2.6	3.1	3.2	5.0	1.6	1.5	1.5

Shown are relative amounts of phosphate monoester (PME),inorganic phosphate (Pi), phosphate diester(PDE), phosphocreatine (PCr), Gamma, Alpha and Beta Adenosine triphosphate (ATP).

TABLE 6. CONTROL 31P NMR SPECTRA, DUNNING -AT TUMOR.

RAT #	VOL (cu. mm.)	PME	Pi	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
2997	1036	14.2	13.9	7.3	13.2	15.8	21.5	14.1
2830	1054	5.5	10.7	1.7	32.0	14.4	20.3	15.4
2939	1137	15.3	18.7	6.8	18.9	10.5	18.0	12.0
2907	1164	14.0	12.9	12.4	13.2	14.2	19.9	13.4
2910	1177	15.1	17.3			12.4	20.0	12.7
2999	1178	16.5	15.5	12.0	9.9	14.6	17.2	14.3
2931	1206	15.2	17.2	12.6	13.4	13.3	17.3	10.9
2930	1277	15.2	14.4	13.2	10.7	12.8	20.6	13.0
2937	1363	11.5	12.9	13.3	12.5	14.8	21.4	13.6
2951	1369	16.1	12.8	14.6	9.1	14.0	18.9	14.5
3000	1419	12.6	10.6	12.2	12.7	17.2	19.6	15.1
2992	1434	14.5	15.3	10.1	12.4	13.3	19.6	14.8
2913	1489	15.7	14.1	7.9	13.4	12.9	20.9	15.0
2994	1535	14.2	15.0	10.0	10.3	13.7	22.1	14.7
2780	1580	13.3	14.6	14.1	14.4	11.3	18.6	13.7
2917	1605	11.0	14.7	8.9	17.4	14.3	20.2	13.6
2964	1636	12.1	17.5	7.4	15.2	14.2	18.8	14.8
2926	1682	15.3	14.4	10.5	11.8	13.9	19.7	14.5
2945	1812	14.2	20.3	13.4	12.8	10.5	17.1	11.8
2912	1942	15.6	12.8	15.0	9.1	15.4	19.6	12.3
2848	4558	16.3	14.4	11.2	16.3	11.2	17.8	12.7
2856	4932	14.6	25.0	7.7	12.7	12.4	18.0	9.7
2919	5117	19.8	17.7	8.9	6.3	13.6	20.7	13.0
2908	6975	13.9	15.0	11.8	11.6	15.2	17.2	15.3
2914	7950	15.1	14.4	14.8	6.8	14.7	19.1	15.2
MEAN:	2305.1	14.3	15.3	10.7	13.2	13.6	19.4	13.6
SD:	1947.2	2.6	3.1	3.2	5.0	1.6	1.5	1.5

Tumors are listed in order of increasing volume. Shown are relative amounts of phosphate monoester (PME),inorganic phosphate (Pi), phosphate diester(PDE), phosphocreatine (PCr), Gamma, Alpha and Beta Adenosine triphosphate (ATP).

TABLE 7. COMPARISON OF CONTROL 31P NMR SPECTRA, SIX SMALLEST AND FIVE LARGEST DUNNING -AT TUMORS.

31P NMR SPECTRA, SIX SMALLEST DUNNING -AT TUMORS.

RAT #	VOL: (cu. mm.)	PME	Pi	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
2997	1036	14.2	13.9	7.3	13.2	15.8	21.5	14.1
2830	1054	5.5	10.7	1.7	32.0	14.4	20.3	15.4
2939	1137	15.3	18.7	6.8	18.9	10.5	18.0	12.0
2907	1164	14.0	12.9	12.4	13.2	14.2	19.9	13.4
2910	1177	15.1	17.3			12.4	20.0	12.7
2999	1178	16.5	15.5	12.0	9.9	14.6	17.2	14.3
MEAN:	1124.3	13.4	14.8	8.0	17.4	13.7	19.5	13.7
SD:	63.5	4.0	2.9	4.4	8.8	1.9	1.6	1.2

31P NMR SPECTRA, FIVE LARGEST DUNNING -AT TUMORS.

RAT #	VOL: (cu. mm.)	PME	Pi	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
2848	4558	16.3	14.4	11.2	16.3	11.2	17.8	12.7
2856	4932	14.6	25.0	7.7	12.7	12.4	18.0	9.7
2919	5117	19.8	17.7	8.9	6.3	13.6	20.7	13.0
2908	6975	13.9	15.0	11.8	11.6	15.2	17.2	15.3
2914	7950	15.1	14.4	14.8	6.8	14.7	19.1	15.2
MEAN:	5906.4	15.9	17.3	10.9	10.7	13.4	18.6	13.2
SD:	1475.6	2.3	4.5	2.8	4.2	1.6	1.4	2.3

Shown are relative amounts of phosphate monoester (PME),inorganic phosphate (Pi), phosphate diester (PDE), phosphocreatine (PCr), Gamma, Alpha and Beta Adenosine triphosphate (ATP). There is a statistically significant difference between the mean volumes of the small and large groups (analysis of variance, Bonferroni t-test, $P<0.001$).

TABLE 8. TREATMENT 31P NMR SPECTRA, DUNNING -AT TUMOR.

RAT #	J/cc	VOL (cu. mm.)	PME	Pi	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
2780	100	1580	13.1	29.9	7.6	13.4	9.2	17.2	9.6
2830	100	1054	10.8	22.4	6.4	23.6	7.8	15.4	13.6
2848	100	4558	11.3	14.5	10.7	13.9	14.8	19.8	14.9
2856	100	4932	11.9	12.8	12.9	18.5	14.3	18.0	11.6
MEAN:		3031.0	11.8	19.9	9.4	17.4	11.5	17.6	12.4
SD:		1996.6	1.0	7.9	3.0	4.8	3.5	1.8	2.3
2910	500	1177	19.6	27.6	7.8	9.3	9.8	15.2	10.6
2913	500	1489	17.4	19.0	10.8	14.0	10.6	16.8	11.5
2917	500	1605	16.0	13.6	8.3	17.3	12.2	19.9	12.6
2907	500	1164	12.4	14.0	11.8	12.9	10.5	18.7	13.7
2912	500	1942	15.6	16.9	13.2	10.9	15.5	17.5	13.5
2908	500	6975	14.6	16.6	14.7	9.2	12.4	19.8	12.7
2914	500	7950	17.2	20.4	7.1	6.9	15.9	16.7	15.7
2919	500	5117	16.8	12.5	15.4	5.5	15.9	19.9	14.0
MEAN:		3427.4	16.2	17.6	11.1	10.8	12.9	18.1	13.0
SD:		2812.4	2.1	4.9	3.2	3.9	2.6	1.8	1.6
2926	1000	1682	16.4	18.8	11.5	15.1	12.9	16.2	9.1
2930	1000	1277	16.2	19.5	8.5	13.0	9.0	19.9	13.8
2931	1000	1206	13.8	18.2	9.7	15.8	12.3	18.9	11.3
2937	1000	1363	14.3	26.2	13.6	12.0	9.5	14.4	10.0
2939	1000	1137	17.6	14.2	9.5	12.8	12.0	19.1	14.8
2951	1000	1369	15.8	11.4	13.1	11.4	14.6	20.4	13.3
2945	1000	1812	10.9	18.7	9.4	14.2	13.6	16.9	16.3
MEAN:		1406.6	15.0	18.1	10.8	13.5	12.0	18.0	12.7
SD:		249.4	2.2	4.6	2.0	1.6	2.1	2.2	2.6
2964	2000	1636	15.5	17.0	10.3	12.9	11.8	19.6	12.7
3000	2000	1419	17.1	13.5	11.5	6.7	16.3	19.8	15.1
2997	2000	1036	15.2	14.0	8.8	10.3	14.5	20.3	16.9
2999	2000	1178	15.0	13.3	11.3	11.1	13.9	19.5	16.0
2994	2000	1535	10.9	16.4	12.8	9.5	16.4	21.5	12.5
2992	2000	1434	13.5	18.4	9.6	12.6	14.0	19.3	12.7
MEAN:		1373.0	14.5	15.4	10.7	10.5	14.5	20.0	14.3
SD:		224.9	2.1	2.1	1.4	2.3	1.7	0.8	1.9

Presenting the light dose at which the tumors were treated (J/cc), and relative amounts of phosphate monoester (PME), inorganic phosphate (Pi), phosphate diester (PDE), phosphocreatine (PCr), Gamma, Alpha and Beta Adenosine triphosphate (ATP). Tumors are grouped according to the total light dose used during PDT.

TABLE 9. COMPARISON OF CONTROL 31P NMR SPECTRA, DUNNING -H AND DUNNING -AT TUMORS.

CONTROL 31P NMR SPECTRA DUNNING -H TUMORS (n=28).

	VOL: (cu. mm.)	PME	Pi	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
MEAN:	1902.4	15.3	8.8	12.7	16.6	13	19.6	14.1
SD:	994.8	2.3	2.4	2.1	3.7	1.7	1.4	1.9

CONTROL 31P NMR SPECTRA DUNNING -AT TUMORS (n=25).

	VOL: (cu. mm.)	PME	Pi	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
MEAN:	2305.1	14.3	15.3	10.7	13.2	13.6	19.4	13.6
SD:	1947.2	2.6	3.1	3.2	5.0	1.6	1.5	1.5

Shown are relative amounts of phosphate monoester (PME),inorganic phosphate (Pi), phosphate diester(PDE), phosphocreatine (PCr), Gamma, Alpha and Beta Adenosine triphosphate (ATP). There are statistically significant differences between the relative amounts of Pi, PDE and PCr present in Dunning -H tumors compared to Dunning -AT tumors (analysis of variance, Bonferroni t-test, $P<0.05$).

TABLE 10. COMPARISON OF 31P NMR SPECTRA FROM DUNNING -AT TUMORS BEFORE PDT SENSITIZED WITH EITHER HEALUX H628 OR PHOTOFRIN II.

CONTROL 31P NMR SPECTRA, DUNNING -AT TUMORS, HEALUX H628 GROUP (n=6).

RAT #	VOL: (cu. mm)	PME	Pi	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
2964	1636.0	12.1	17.5	7.4	15.2	14.2	18.8	14.8
3000	1419.0	12.6	10.6	12.2	12.7	17.2	19.6	15.1
2997	1036.0	14.2	13.9	7.3	13.2	15.8	21.5	14.1
2999	1178.0	16.5	15.5	12.0	9.9	14.6	17.2	14.3
2994	1535.0	14.2	15.0	10.0	10.3	13.7	22.1	14.7
2992	1434.0	14.5	15.3	10.1	12.4	13.3	19.6	14.8
MEAN:	1176.9	12.0	12.5	8.4	10.5	12.7	17.0	12.5
SD:	224.9	1.6	2.3	2.1	2.0	1.5	1.8	0.4

CONTROL 31P NMR SPECTRA, DUNNING -AT TUMORS, PHOTOFRIN II GROUP (n=4):

RAT #	VOL: (cu. mm)	PME	Pi	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
3080.0	1745.0	14.7	31.3	7.9	12.1	8.5	16.3	9.2
3112.0	1563.0	11.8	18.1	12.1	12.6	14.0	19.5	11.9
3115.0	1078.0	14.7	17.7	12.7	14.9	11.4	16.8	11.8
3092.0	1236.0	16.5	21.4	11.0	12.5	8.8	17.6	12.2
MEAN:	1405.5	14.4	22.1	10.9	13.0	10.7	17.6	11.3
SD:	303.3	1.9	6.3	2.1	1.3	2.6	1.4	1.4

Shown are relative amounts of phosphate monoester (PME),inorganic phosphate (Pi), phosphate diester(PDE), phosphocreatine (PCr), Gamma, Alpha and Beta Adenosine triphosphate (ATP), for both Healux H628 and Photofrin II experimental groups.

Table 11. COMPARISON OF 31P NMR SPECTRA FROM DUNNING -AT TUMORS AFTER PDT SENSITIZED WITH EITHER HEALUX H628 OR PHOTOFRIN II.

POST-TREATMENT 31P NMR SPECTRA, DUNNING - AT TUMORS, HEALUX H628 GROUP (n=6).

RAT #	J/cc	VOL: (cu. mm.)	PME	Pi	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
2964	2000	1636.0	15.5	17.0	10.3	12.9	11.8	19.6	12.7
3000	2000	1419.0	17.1	13.5	11.5	6.7	16.3	19.8	15.1
2997	2000	1036.0	15.2	14.0	8.8	10.3	14.5	20.3	16.9
2999	2000	1178.0	15.0	13.3	11.3	11.1	13.9	19.5	16.0
2994	2000	1535.0	10.9	16.4	12.8	9.5	16.4	21.5	12.5
2992	2000	1434.0	13.5	18.4	9.6	12.6	14.0	19.3	12.7
MEAN:		1373.0	14.5	15.4	10.7	10.5	14.5	20.0	14.3
SD:		224.9	2.1	2.1	1.4	2.3	1.7	0.8	1.9

POST-TREATMENT 31P NMR SPECTRA, DUNNING - AT TUMORS, PHOTOFRIN II GROUP (n=4).

RAT #	J/cc	VOL: (cu. mm.)	PME	Pi	PDE	PCr	GAMMA ATP	ALPHA ATP	BETA ATP
3080.0	2000	1745.0	17.9	47.6	3.4	11.4	4.7	10.4	4.7
3112.0	2000	1563.0	15.1	52.8	4.6	9.4	5.7	8.9	3.5
3115.0	2000	1078.0	13.5	37.4	7.5	12.1	8.0	14.6	6.9
3092.0	2000	1236.0	14.7	49.2	5.9	9.8	6.1	10.7	3.6
MEAN:		1405.5	15.3	46.8	5.4	10.7	6.1	11.2	4.7
SD:		303.3	1.9	6.6	1.8	1.3	1.4	2.4	1.6

Shown are relative amounts of phosphate monoester (PME), inorganic phosphate (Pi), phosphate diester (PDE), phosphocreatine (PCr), Gamma, Alpha and Beta Adenosine triphosphate (ATP).

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